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Open reference resource for genomic copy number aberrations in cancer implementing emerging global standards

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Genomic copy number variations (CNV) represent the majority of the mutational landscape in the most malignancies. While specific CNV events and some recurring patterns have contributed to the identification of individual cancer drivers and the recognition of cancer subtypes, the complexity of genomic CNV patterns requires large amounts of well-defined genomic profiles.

The “Beacon” protocol - developed with support from ELIXIR, the European bioinformatics infrastructure organization, as a standard of the Global Alliance for Genomics and Health (GA4GH) - represents an emerging standard for an “Internet for Genomics”. While the initial version of the protocol served as a widely adopted test bed for the sharing of genomic variants over federated query systems connecting hundreds of internationally distributed resources, the version 2 of the protocol provides a framework for extended, metadata-rich query and response options in both public and restricted federated access scenarios. Throughout the development of Beacon v2, the Progenetix cancer genomics resource (progenetix.org) - including the largest publicly accessible set of cancer CNV data - has served as a driver for Beacon v2 protocol features.

With the implementation of the Beacon v2 API as backbone of the Progenetix resource - serving genome-wide CNV profiling data from more than 130’000 individual experiments, representing over 700 diagnostic entities and more that 1’500 published studies - we could demonstrate the use of the GA4GH Beacon v2 protocol as a practical solution for the sharing of vast amounts of genomic profiling data, in a open setting and using a documented, completely open API for third party use.

With the Beacon v2 under review as GA4GH standard, work is focusses on a wide set of use cases, from clinical information systems to public-access research databases. Importantly, an ELIXIR-supported implementation study by members of the ELIXIR h-CNV community (cnvar.org) will explore the Progenetix scenario for various European genomics resources, in human genetics / rare diseases and cancer genomics.

In summary, the Progenetix resource provides the largest reference resource for copy number variations in cancer, with an open access protocol based on emerging, international reference standards for integration into diagnostic and pharmacogenomic analysis workflows.

Keywords :
cancer, genomics, standards CNV, copy number variations

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Fatty acid synthase as a novel prognostic biomarker and a potential therapeutic target in diffuse malignant peritoneal mesothelioma

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Introduction

Diffuse malignant peritoneal mesothelioma (DMPM) is a rare and locally aggressive tumor with a dismal prognosis. The availability of new therapies and biomarkers for patients' selection is still an urgent need. Altered lipid metabolism, which has been reported in several tumors, has not yet been studied in DMPM. By comparatively analyzing gene expression profiles of relapsed and non-relapsed DMPM tumors, we found the up-regulation of fatty acid synthase (FASN) in relapsed tumors. In this study , we investigated the prognostic role of FASN in a cohort of DMPM patients and assessed the antitumor effects of FASN inhibitory strategies in patient-derived DMPM cell lines.

Materials and methods

FASN expression was assessed in clinical samples from 80 DMPM patients by immunohistochemistry and its prognostic role was evaluated using Cox regression analysis. To validate FASN as a therapeutic target, the cytotoxic effects of FASN inhibitors (orlistat, cerulenin and C75), as single agents and in combination with Selinexor -a selective inhibitor of the exportin chromosome maintenance protein-, were investigated on DEX and MP115 cell lines. Drug interaction was analyzed by Chou and Talalay method. Treatment-induced apoptosis and cell cycle perturbations were assessed by western blotting and flow cytometry, respectively.

Results

The log-rank test indicated that high FASN staining was significantly associated with a reduced Relapse-Free Survival (RFS) (HR: 1.144; p=0.014) and Overall Survival (OS) (HR: 1.854; p=0.032) in DMPM patients. FASN prognostic value was also maintained in multivariate analysis in terms of both RFS (HR: 2.240; p=0.007) and OS (HR: 2.246; p=0.014). FASN pharmacological inhibition in DMPM cells resulted in a significant reduction of cell proliferation, especially upon C75 exposure. In combination experiments, a synergistic activity of FASN inhibitors and selinexor was also observed. In addition, compared to single agents, combined treatments resulted in an increased apoptosis induction and cell cycle impairment, as indicated by caspase-dependent apoptosis activation and cell accumulation in the G2-phase.

Conclusion

Overall, our results support a role of FASN as i) a prognostic biomarker for DMPM patients and ii) a novel therapeutic target for the disease.

Role of sphingolipid metabolism in osimertinib-resistance in EGFR-mutated NSCLC models

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Introduction

The third-generation mutant-selective EGFR tyrosine kinase inhibitor osimertinib is approved for the first-line treatment of EGFR-mutated Non-Small Cell Lung Cancer (NSCLC) patients. Despite the efficacy of the drug, patients develop resistance. The mechanisms of osimertinib-resistance are heterogeneous and not fully understood, and their characterization is essential to develop new therapeutic strategies to delay or overcome resistance.
Emerging evidences indicate a role of lipid metabolic reprogramming in lung cancer development, progression and resistance to TKI-treatment.

Materials and methods

Experiments were performed in PC9 and PC9T790M osimertinib-sensitive (OS) EGFR-mutant NSCLC cell lines and in their derived osimertinib-resistant (OR) clones. Cell lipidome was profiled by UHPLC_MS. Cell proliferation, cell death, colony formation, cell migration have been evaluated.

Results

The lipidome analysis showed a significant difference between the lipidome of OS and OR cells, displaying a reprogramming in sphingolipid pathway in OR vs OS cells. In particular, an increase in intracellular content of (poli)glicosylceramides were observed in OR cells in comparison with OS cells. In OR cells the inhibition of GCS by PDMP caused cell cycle arrest, inhibition of 2D and 3D cell proliferation, colony formation, cell migration and cell death induction. PDMP inhibited cell proliferation also in the OS cells, but did not affect the viability of normal lung cells. In OS cells the combination of PDMP with osimertinib significantly enhanced the inhibition of proliferation and the induction of apoptosis compared to single treatments. Very interestingly, in OS cells the addition of PDMP prevented the onset of resistance to osimertinib in long-term experiments, through the eradication of the persistent drug-tolerant cell subpopulation.

Conclusion

The accumulation of GlcCers has been linked to the development of drug resistance in different types of cancer and a higher content of GlcCer was observed in malignant lung pleural effusions from NSCLC patients. Our findings indicate a role of the dysregulation of ceramide metabolism in the mechanism of resistance to osimertinib, and suggest that targeting GCS may be a promising therapeutic strategy to treat EGFR-mutant NSCLC patients progressed to osimertinib. Moreover, the inhibition of GCS combined with osimertinib in first-line treatment may be a strategy to prevent osimertinib-resistance.

B-raf inhibitors and diclofenac target glycolytic metabolism of BRAFmutated thyroid carcinomas and synergistically restrain cell viability

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Introduction and Objectives

Although B-raf inhibitors (BRAFi) are effective drugs for BRAF-mutated papillary (PTC) and anaplastic (ATC) thyroid carcinomas, acquired resistance can limit drug efficacy [1]. Metabolic reprogramming provides tumor cells proliferative adaptation to anti-tumor therapies and targeting tumor metabolism is emerging as a powerful approach [2,3]. We exploited metabolic vulnerabilities of BRAF-mutated thyroid tumors and propose combinatorial approaches to maximize the efficacy of approved BRAFi.

Methods

Tumor subtype-specific expression and methylation patterns were identified for BRAF-mutated tumors using multiple omic data. Key transcription factors were selected by screening metabolic genes' promoters. HIF1A silencing and stabilization was performed in BCPAP cells by siRNAs and CoCl2, respectively. The analysis of gene expression, glucose uptake, lactate efflux and cell viability were performed in BCPAP, 8505c and Nthy-3-ori cells treated with BRAFi (i.e. vemurafenib, VMR) and a non-steroidal anti-inflammatory drug (i.e. diclofenac) showing anti-metabolic properties [3,4].

Results

A metabolic gene signature characterized by increased glucose uptake and lactate efflux was identified as a hallmark of BRAF-like thyroid tumors. Metabolic genes' signature is not fully recapitulated by CpG islands' methylation changes, whereas HIF1A was identified as a key regulator of energy metabolism-related genes. Both MAPK activation and HIF1A stabilization increase the levels of selected metabolic genes, whereas B-raf inhibition (by VMR) and HIF1A knockdown have repressive effects. Moreover, HIF1α stabilization counteracts VMR effects on energy metabolism-related genes and tumor cell viability.

Notably, both VMR and diclofenac restrain the glycolytic phenotype of BRAF-mutated tumor cells and their combination synergistically reduce tumor cell viability.

Conclusion

We highlight a new metabolic vulnerability of BRAF-mutated thyroid carcinomas and assess the synergistic ability of BRAFi and diclofenac to restrain cell viability, potentially opening new interesting therapeutic perspectives especially for drug resistant PTCs and ATCs.

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Insight on pancreatic cancer nerves: uncovering the cross-talking between TGFβ and NGF and their impact on clinical outcomes

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is notable for its chemoresistance, poor prognosis and debilitating pain. One of the hallmarks of PDAC is the desmoplastic stroma. Stromal components can contribute to perineural invasion (PNI), a predictor of poor prognosis associated to cancer pain. TGFβ plays a role in stromal plasticity and recent evidences suggest that it could be released by nerves, increasing the expression of nerve growth factor (NGF) by PDAC cells. Further studies should assess the relationship between neuronal and PDAC cells and potential pharmacological intervention to influence PNI and survival outcomes.

Materials and methods

To evaluate the source of TGFβ and NGF, we performed specific ELISAs on the neuronal-like pheochromocytoma PC12 cell line and on human PDAC cells PANC1, BxPC3 and primary cultures PDAC2 and pp161. Inhibition of cell growth was evaluated by sulforhodamine B assay, testing galunisertib, a TGFβRI inhibitor, alone or in combination with gemcitabine, while cell migration was assessed using wound-healing assay. These experiments were replicated exposing PANC-1 cells to the conditioned medium (CM) of PC12. In addition, we studied the effects of PANC-1 CM on PC12. The expression levels of NGF and of SMAD2, a TGFβ signaling mediator, were studied by immunohistochemistry in Tissue Microarrays (TMAs) to establish a correlation with survival, PNI and metastatic phenotype.

Results

ELISA revealed that TGFβ is released mainly by PC12, and no cytotoxic nor antimigratory activity was observed for galunisertib. Conversely, exposure of PANC-1 cells to PC12 CM and treatment with galunisertib resulted in a significantly reduced proliferation and IC50 for the combination with gemcitabine. Similarly, the wound-healing assay demonstrated an antimigratory effect of galunisertib on PANC-1 exposed to PC12 CM. An axonal-like elongation was observed when PC12 were exposed to PANC-1 CM. Additionally, high expression levels of NGF and SMAD2 in TMAs were associated with a significantly shorter OS, and SMAD2 expression was correlated with PNI.

Conclusions

Our study demonstrates a cross-talking between PDAC and neuronal cells, providing new insight into the mechanisms underlying PNI. NGF and SMAD2 emerged as poor prognostic factors predicting outcome of PDAC patients and might guide personalized medicine with drugs targeting the interaction between tumor cells and nerves.

Investigation into Antigrowth Effect of Palladium(II)-Barbiturate Complex on Different Genetic Subtypes of Colon Cancer

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Colorectal cancer (CRC) is the second most common cancer type worldwide. Treatment modalities and different specific molecule blockers are insufficient to cure mutated CRC tumors. Resistance to these medications has been connected to downstream mutations such as KRAS, BRAF, and NRAS, which result in repetitive activation of EGFR downstream targets and also bypass EGFR blockage approaches. Palladium complexes' anticancer activities have recently received much more attention due to their chemical resemblance to platinum-based compounds. The aim of this study was to evaluate the antigrowth/cytotoxic effect of newly synthesized Pd(II) complex to provide an effective treatment modality for aggressive KRAS and BRAF mutant colorectal cancer cells.

The anti-growth effect of the Pd(II) dinuclear complex was evaluated on HCT15 and HT29 cell lines using luminescent ATP assay. Mode of cell death was evaluated by flow cytometry (for translocation of phosphatidylserine, caspase3/7 activation, DNA damage, reactive oxygen species level, and Bcl-2 expression and activation), and western blotting. Moreover, the antimigration property of Pd(II) complex was elucidated by vertical/horizontal migration and tube formation tests.

Pd(II) complex exhibited an anti-growth effect against both HCT15 and HT29 cells in a dose (0.38–1.75 μM) and time (12, 24, 48 h) dependent manner. Apoptosis was evidenced by phosphatidylserine translocation and caspase 3/7 activation. Additionally, Pd(II) complex downregulated the phosphorylated forms of MAPK pathway-related proteins (such as ERK1/2 and MEK1/2) and decreased the mobility of HT29 and HCT15 cell lines as demonstrated by migration assays.

In conclusion, the Pd(II) complex shows promise as a possible treatment option for aggressive colorectal tumors with KRAS and BRAF mutations, which are currently being researched in animal models by our group.

Keywords:
Chemotherapy, Palladium, Invasion, Anti-cancer, HUVEC, Caspase3/7, wound healing, Colon Cancer

Early Pharmacological Profiling of Antiproliferative Compounds by Continuous Live-Cell Imaging

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The identification of drug targets remains a major challenge in drug discovery. The challenge in a phenotypic drug discovery strategy is to fully understand and elucidate the mode of action, in addition to the resolution of the mechanism of action (MoA), identifying the molecular target(s) affected(s) by the molecule and responsible for its pharmacological activity. Fortunately, this complexity reduces substantially with the development of advanced testing technologies and bioinformatics tools that make phenotypic drug discovery an accessible and realistic strategy. Despite this, there is no established general methodology and the scientific literature on this transcendental topic is scarce and atomized. Studies concerning the in silico prediction of MoA to date rely on data obtained from costly experimental measurements.

In this work, we explored the combination of label-free continuous live-cell imaging and machine learning techniques as a promising tool to depict in a fast and affordable test the mode of action of small molecules with antiproliferative activity. To develop the model, we selected the non-small cell lung cancer cell line SW1573, which was exposed to the known antimitotic drugs paclitaxel, colchicine and vinblastine. The novelty of our methodology focuses on two main features with the highest relevance, (a) meaningful phenotypic metrics, and (b) fast Fourier transform (FFT) of the time series of the phenotypic parameters into their corresponding amplitudes and phases. The resulting algorithm was able to cluster the microtubule disruptors, and meanwhile showed a negative correlation between paclitaxel and the other treatments. The FFT approach was able to group the samples as efficiently as checking by eye. This methodology could easily scale to group a large amount of data without visual supervision.

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A reverse molecular pharmacology approach to unveil new therapeutic options in high-risk neuroblastoma

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Introduction

High-risk neuroblastoma (NB) is one of the deadliest forms of childhood cancer. Despite considerable research efforts to develop safer and more effective treatments, a significant proportion of NB patients will not respond to current therapeutic options. In this study, we addressed the questions of how can new therapeutic targets be quickly identified and what role(s) they play in NB biology and drug resistance.

Method

We developed an original strategy using pharmacogenomics to discover new targets in NB. This method is based on high-throughput drug screening coupled with in-depth chemoinformatics and transcriptomic analyses.

Results

Our method revealed 66 non-toxic drugs that could re-sensitize multi-drug resistant NB cells to standard-of-care. Amongst the 195 putative targets of those hit compounds, 7 were found to be associated with a worse clinical outcome when highly expressed at the gene level in neuroblastoma tumors. A siRNA screening targeting those 7 genes was performed to identify the most promising factor regulating NB progression and drug resistance. Our method revealed Interleukin-1 Receptor-Associated Kinase 1 (IRAK1), which is involved in diverse cellular processes including inflammation and innate immunity, as the top gene hit. Our validation experiments ascertained that IRAK1 knockdown decreased NB cell proliferation by 30-40 % in 2D and 3D models. Moreover, we showed that silencing IRAK1 expression enhanced the cytotoxic activity of vincristine in NB cells. A pharmacological study combining IRAK1 small molecule inhibitor with vincristine also induced a 4 to 10-fold increase in cytotoxicity in 10 out of 12 tested NB cell lines. Finally, by functional genomics using RNA interference and overexpression plasmids, our results revealed a complex relationship between the different IRAK family members to regulate NB cell proliferation and response to chemotherapy.

Perspectives

By developing an original method combining a high-throughput drug screening based on repurposing principles with chemo-bio-informatic analyses and functional genomics, our data revealed, for the first time, IRAK1 as a novel target in high-risk NB. Thus, by unraveling the oncogenic role of IRAK1 immune signaling pathway and deciphering its implication in drug resistance, our results could improve our understanding of NB biology and open innovative therapeutic avenues for NB patients.

Keywords:
Neuroblastoma, IRAK1, drug resistance, functional genomics

Combining sunitinib and anti-PD1 nivolumab is a valid strategy to enhance anti-cancer immunity and therapeutic potential in CRC

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Colorectal cancer (CRC) is a leading cause of cancer death worldwide. Anti-angiogenic drugs and chemotherapy represent a standard of care for the metastatic disease. Immunotherapy with checkpoints inhibitors (ICIs) represents the new era of CRC treatment, though effective response is restricted to patients with mismatch repair-deficient (dMMR) and/or microsatellite instability-high (MSI-H) tumors. Emerging evidence suggests that anti-angiogenic drugs could exhibit immunomodulatory properties, thus suggesting combination of ICIs with angiogenesis-targeting agents. In this study we evaluated the ability of sunitinib in reducing macrophages mediated-immunosuppression and in inhibiting tumor growth when combined with anti-PD1 nivolumab.

Material and Methods

We used CaCo-2, Colo205, LoVo and HT-29 CRC cell lines and six patient-derived organoids (PDOs) diagnosed with CRC for in vitro and ex vivo evaluations. The cytotoxicity of treatments was evaluated by MTT assay in the CRC cell lines and by Cell Titer-Glo® 3D Cell Viability Assay in PDOs, in presence of authologus pre-activated T cells obtained from each patient. Apoptosis induction was determined by propidium iodide/AnnexinV staining and NucView® 488 Caspase-3 Assay Kit. The migration of tumor associated macrophages (TAMs) was determined in a co-culture in vitro assay. TAMs polarization was assayed by flow cytometry. Western blotting was used to determine the effects of treatment(s) on target proteins in TAMs.

Results

The combined treatment resulted in a synergistic inhibition of cell viability in the majority of CRC models and in strong reduction of the viability of PDOs, by causing apoptosis through caspase-3 activation. In CRC–conditioned macrophages, the combined treatment prevented the M0-to-M2 polarization by inducing the reduction of Arginase 1+ TAMs and promoted instead the M0 to M1 polarization. Additionally, the combined treatment promoted the recruitment of M1 and prevented that of M2 macrophages, thereby supporting the reduction of immunosuppressive tumor microenvironment. Notably, the treatments affected the activity of unfolded protein response (UPR) pathways, such as IRE/XBP-1, which potentially play a role in the polarization of myeloid cells.

Conclusions

Overall our results provided translational evidence and mechanistic insights that may pave a reliable path for experimentation that optimize the use of sunitinib in combination with immunotherapy for CRC.

Keywords:
CRC, anti-angiogenic agents, anti-PD1, macrophages

Gender-related metabolic differences in melanoma: correlation with an immunosuppressive environment

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Introduction

The epidemiology of melanoma has highlighted sexual dimorphism with a higher incidence and mortality rate in the male population than in the female one [1-2]. In addition, gender disparity in immune response has been highlighted as a key point in cancer incidence, outcome and response to therapy [3]. In recent decades, metabolic alterations in the tumor microenvironment have been shown to affect the balance between immune surveillance and immune escaping, thus leading to an anti-tumor or pro-tumor immune response [4]. It's well recognized that melanoma exhibits an increased glycolytic metabolism, based on a high glucose intake and on upregulation of several glycolytic enzymes such as Hexokinase II (HKII) and lactate dehydrogenase A (LDH-A), resulting in a lactate-rich microenvironment. However, there are no clear evidence in melanoma about gender-related differences in the metabolic profile and their possible impact on the reshaping of the immune infiltration. In this scenario, we aim to better investigate whether gender differences can affect the metabolic signature of melanoma and how this impact on the composition of the immune infiltration.

Result

First, we performed a metabolic analysis by a SeaHorse analysis of representative male and female melanoma cell lines, observing higher levels of hexokinase II (HKII) and lactate dehydrogenase-A (LDH-A) in male cell lines, accompanied by a more pronounced secretion of lactate. In keeping, an IHC analysis performed on tissue microarrays (TMA) confirmed higher LDH-A expression levels in male melanoma samples compared to female ones, accompanied by a more pronounced CD4+ infiltration. Interestingly, we also observed an enrichment in the immune suppressive Treg population in male melanoma cells co-cultured with CD4+ cells as well as in male melanoma specimens. The impairment of LDH-A expression in male melanoma cells by a silencing approach or the treatment with the LDH-A selective inhibitor FX11 showed an alteration of the immune cell composition, with a significant reduction in the Treg percentage.

Conclusion

Our data provide evidence of a strong correlation between the metabolic profile of melanoma and the reshaping of the immune infiltration in a gender-dependent context and support the role of lactate in eliciting an immunosuppressive landscape in male melanoma.

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The impact of the efflux transporter ABCG2 gene polymorphism on the development of adverse events in CML patients treated with Imatinib

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Introduction

Imatinib is the first-line treatment for chronic myeloid leukemia (CML). Genetic variations in the efflux transporters such as ABCG2 may play important roles in the safety of Imatinib. Polymorphism of ABCG2 gene may act as a good predictor for response and toxicity of Imatinib in CML Egyptian patients. The purpose of the present study was to investigate the association between ABCG2 gene polymorphism with the Imatinib adverse effects in CML patients.

Materials and methods

One hundred and two patients with CML at chronic phase were recruited in this study. Genetic polymorphism of the genes ABCG2 SNPs 34 G>A was studied using PCR-RFLP technique. The relationship was examined between ABCG2 SNPs 34 G>A gene polymorphism and dizziness as well as headache which are two of the most frequently occurring adverse effects of Imatinib.

Results

Forty-seven of the participants were males with average ages of 40.6 ±11years. While 29 patients (28.4%) experienced no adverse events, dizziness, headache were detected as two of the most prevalent adverse events of Imatinib. Of our patients, 81.4% had the wild-type GG allele of the efflux transporter ABCG2.34 G>A gene, 16.7% had the heterozygous GA allele, and 2% had the variant type AA. This study found a statistically significant relation between ABCG2.34G>A and dizziness as well as headache frequency ($P<0.001$). After carrying out binary logistic regression analyses, no significant risk factors were detected in relation to the occurrence of headache. Regarding the occurrence of dizziness, we reported that the occurrence of dizziness increased by 2.3 times in patients with heterozygous GA allele of the ABCG2.34 ($P<0.001$) compared to homozygous wild type GG.

Conclusion

Our observation emphasizes that, Imatinib may cause dizziness and headache. Also, it was found that the ABCG2.34 G>A gene polymorphism has an effect on the occurrence of dizziness and headache in patients treated with Imatinib as well as its effect on the response of Imatinib as previously mentioned. Finally, it can be concluded that therapeutic drug monitoring and Pharmacogenetic screening are required to improve Imatinib therapy management.

Keywords:
Imatinib, Chronic myelogenous leukemia, Polymorphism, Adverse effects

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Identification of novel strategies to combat ABCB1 gene amplification as a key mechanism underlying paclitaxel resistance in PDAC cells

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest types of cancer. Most trials using targeted agents have been unsuccessful and efficacy of chemotherapies such as gemcitabine and nab-paclitaxel (nab-PTX) is limited by intrinsic or acquired resistance. Mechanisms of resistance are poorly understood. ATP-binding cassette (ABC) transporters play a major role in cancer drug resistance but have not been implicated in PTX-resistance of PDAC. The aim of this study was to investigate the role of ABC transporters in PDAC PTX-resistance and explore strategies to overcome it.

Methods

We established three paclitaxel resistant (PR) PDAC cell lines using Patu-T, Suit2.007 and Suit2.028. Transcriptomics and proteomics datasets were generated for Patu-T PR and Suit2.028 PR. The role of ABCB1 in PR cells was assessed by pharmacological inhibition with verapamil and siRNA-mediated gene silencing. RT-qPCR of genes in the ABCB1 locus and detection of extrachromosomal DNA (ecDNA) were used to investigate gene amplification. Kinase-inhibitor (KI) library screening and SRB assays were performed to identify synthetic lethalities with PTX in PR cells.

Results

Transcriptomics and proteomics showed significant upregulation of ABCB1 in Patu-T PR and Suit2.028 PR and this was validated by RT-qPCR and Western Blot in all three cell lines. Verapamil strongly sensitized the three PR models to PTX, and siRNA-mediated depletion further established the key role of ABCB1 in PTX resistance. Concomitant upregulation of ABCB4, ADAM22 and TP53TG1 pointed to ABCB1 locus amplification, which did not involve emergence of ecDNA. Importantly, we identified known and novel KIs that sensitize PR PDAC cells to PTX through ABCB1 inhibition or other, as yet unknown mechanisms.

Conclusion

We identify upregulation of ABCB1 through locus amplification as a common mechanism of PTX-resistance in PDAC cells. As toxicity has hampered clinical use of ABC-transporter inhibitors in the context of solid tumors, the KIs identified in this study are promising candidates for further exploration as therapeutic strategies to overcome PDAC PTX-resistance.

Standardized immunohistochemical and pharmacological studies to evaluate Human Equilibrative Nucleoside Transporter 1 (hENT1) as a predictive and prognostic marker in patients with cholangiocarcinoma

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Background

Cholangiocarcinoma (CCA) is a lethal tumor with a 5-year survival of less than 10%. Effective (neo)adjuvant chemotherapy is lacking due to resistance to chemotherapy, including gemcitabine, and absence of predictive markers. Human equilibrative nucleoside transporter 1 (hENT1) is known as a gemcitabine transporter and has been described as a potential prognostic and predictive biomarker for pancreatic cancer, but few studies investigated its role in cholangiocarcinoma. Hence, we compared rabbit-derived (SP120) and murine-derived (10D7G2) antibodies for their potential to detect hENT1 expression in perihilar CCA (pCCA) tissue, and we explored the predictive value of hENT1 in three extrahepatic CCA cell lines.

Methods

Tissues of 71 chemonaïve patients with histological confirmation of pCCA were retrospectively selected and stained with SP120 or 10D7G2 in order to evaluate 1) the inter-observer variability for both antibodies and 2) the correlation with overall survival (OS). Concomitantly, three extrahepatic cholangiocarcinoma cell lines (EGI-1, TFK-1 and SK-Cha-1) were used to assess gemcitabine sensitivity after hENT1 knockdown, as assessed by sulforhodamine-B assay.

Results

When SP120 was used, 15.5% of the tumor samples were positive and 60.6% were negative for hENT1 expression, while when 10D7G2 was used, 22.5% samples were positive and 29.6% negative. The Cohen's kappa for SP120 and 10D7G2 was 0.845 (high-agreement) and 0.485 (moderate-agreement), respectively. For SP120, the median OS was 41 months in hENT1 positive and 36 months in hENT1 negative patients (P=0.780). For 10D7G2, the median OS was 58 months in hENT1 positive and 19 months in hENT1 negative patients (P<0.001). In the cell lines, significantly increased IC50 values were found in SK-ChA-1 and TFK-1 cell lines after hENT1 knockdown (P<0.05).

Conclusion

Scoring immunohistochemistry (IHC) for hENT1 expression with the use of SP120 antibody resulted in the highest inter-observer agreement but did not show a prognostic role of hENT1. However, a prognostic role was observed when using 10D7G2. Moreover, we found a potential predictive role for gemcitabine sensitivity in hENT1 in SK-ChA-1 and TFK-1 cells. This prompts further studies for both IHC standardization and preclinical validation of the role of hENT1 in cholangiocarcinoma patients treated with gemcitabine-based chemotherapy.

Cytotoxic activity of novel 1,2,4-oxadiazole-based derivatives in pancreatic ductal adenocarcinoma by modulation of CDK1 and GSK3β

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Introduction and objectives

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with increasing incidence and poor prognosis due to its late diagnosis and intrinsic chemoresistance. These features pose a series of therapeutic challenges and new targets are needed [1]. Protein kinases have been investigated as promising targets given their role in fundamental cellular activities [2]. Aberrant activation of cyclin-dependent kinases (CDKs) and glycogen synthase kinase-3-beta (GSK3β) are hallmarks of PDAC. Thus, we decided to synthesize novel 1,2,4-oxadiazole-based derivatives as CDK1 and GSK3β modulators and evaluate their pharmacological activities in preclinical models of PDAC.

Methods

Sixteen bis(indolyl)1,2,4-oxadiazoles were synthetized, evaluated for their antiproliferative activity in mesenchymal and epithelial PDAC cells, by Sulforhodamine-B and wound-healing assays. Kinase-arrays and ELISAs were employed to test the modulation of oncogenic kinases. Structural manipulation through the replacement of an indole portion with a 7-azaindole ring led to the synthesis of ten novel indolyl-oxadiazole-7-azaindoles, which were tested for their antitumor activities as well as for their ability to reduce CDK1 expression and induce apoptosis.

Results

The new bis(indolyl)oxadiazoles exhibited antiproliferative activity against various PDAC cells, including SUIT-2, Capan-1, and PANC-1, eliciting EC50 values in the sub-micromolar range, associated with significant reduction of migration. These results might be explained by the effects on epithelial-to-mesenchymal transition markers, including SNAIL-1/2 and metalloproteinase-9. Moreover, cytometry after Annexin V-FITC and propidium-iodide staining demonstrated apoptosis induction. Keeping with these data, PathScan and ELISA arrays revealed cleavage of caspase-3 and PARP and a significant inhibition of GSK3β phosphorylation, suggesting this kinase as the main downstream target. The derivatives bearing a 7-azaindole ring showed similar antiproliferative and pro-apoptotic activity (increased by 2-fold in the primary cell cultures PDAC3 cells) and the ability to inhibit CDK1 expression. Lastly, these compounds passed the ADME prediction, showing good pharmacokinetic parameters.

Conclusion

Novel series of 1,2,4-oxadiazoles have been successfully synthetized and showed their ability to reduce cancer growth, migration and induce apoptosis. The pharmacological potential of these compounds was confirmed by their ability to modulate the enzymatic activity of GSK3β and CDK1, supporting future studies for their development as potential personalized medicine approaches against PDAC.

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GLUT-1: a new target/prognostic factor in Hepatobiliary cancers?

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Introduction

In several tumors, higher GLUT-1 expression is related to significantly poorer prognosis. In particular, the correlation between increased GLUT-1 expression and shorter overall survival (OS) has been already reported for pancreatic ductal adenocarcinoma and intrahepatic cholangiocarcinoma, but not yet for extrahepatic cholangiocarcinoma (EC). Therefore, the aim of our study was to evaluate the correlation between tissue expression of GLUT-1 and OS in EC. Furthermore, we evaluated the activity of novel compounds targeting GLUT-1 in EC-derived cell lines.

Methods

We obtained tissues from n=23 patients undergoing radical resection and built tissue microarrays (TMA) to assess GLUT-1 expression by immunohistochemistry, evaluating both the intensity and the percentage of cells expressing this transporter. The antiproliferative activity of the novel GLUT1 inhibitors PGL-10, PGL-11, PGL-13, PGL-14, PGL-17, PGL-18 and PGL-27 in the EC cells EGI1 and TFK1 was assessed using the sulforhodamine-B assay. SPSS-IBM-26 software was used for statistical analysis.

Results

Patients with high GLUT-1 expression had a significantly shorter OS compared to patients with low GLUT-1 expression (25.5 vs. 50.3 months, p=0.045).

PGL14 (GLUT-1 inhibitor) showed antiproliferative activity in EC cells comparable to the promising activity demonstrated in PDAC cells. The IC50s ranged from 13.9 to 32.0 uM after 72-hour exposure. Notably, these compounds were still active in 3D spheroids of these tumor cells, which showed increased resistance to gemcitabine and are more representative of tumor tissues because of the hypoxic areas, as detected by immunohistochemical stainings.

Remarkably, the GLUT-1 inhibitor compound showed a synergistic interaction with gemcitabine, increasing apoptosis induction and accumulation of ROS. Furthermore, the combination of these drugs reduced cell migration and enhanced in vitro sensitivity to anoikis, suggesting the ability of these compound to inhibit metastasis.

Conclusions

These results support the role of GLUT-1 as a promising prognostic factor in EC patients and opens the way for new studies on the effectiveness of GLUT-1 inhibitors. Additional studies to characterize the effects of these compounds on invasiveness and pathways involved in tumor-metastasis interaction are warranted.

Keywords:
Hepatobiliary cancer, target therapy

Adaptive-dosing strategies of high dose Busulfan in bone-marrow transplant conditioning

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Introduction

High-Dose Busulfan is widely used to trigger aplasia in leukemia patients scheduled for allogenic bone marrow transplant. Target AUCs of 16000 µM.min or 20000 µM.min are usually considered as the exposure to be reached to ensure an optimal toxicity/efficacy ratio. HD Busulfan is administered as 4 to 6 sequential courses the previous days before transplant. Although effective, this regimen is associated with life-threatening toxicities due to a large interindividual pharmacokinetics variability and reducing treatment-related toxicities is still an ongoing issue.

Methods

we have implemented a population-based strategy to identify individual PK parameters of HD Busulfan during the first course of the conditioning, i.e., when Busulfan was administered following standard dosing. Busulfan was assayed following a sparse-sampling strategy (i.e., T3h15, T9H, T 12H) by mass spectrometry method fully validated following EMA standards. Using a WLSQ estimator, and PK parameters computed from 14 adult patients previously treated with HD Busulan, it was possible to estimate PK parameters and exposure metrics. Assuming that clearance remains stable throughout the successive courses, it was thus possible to personalize the dosing for the next administrations. Exposure was finally checked during the last course, by mass spec analysis and AUC calculation.

Results

Thirty patients were monitored and had their Busulfan dosing adapted. Busulfan PK was best described by a one-compartment model. A wide inter-patient variability was observed and absolute deviation from the target exposure was comprised between 10 to 40%. Accordingly, dosing was modified over a -40 to +40% range as compared with standard dosing. After adaptive dosing, final deviation from the target exposure was reduced below 10%, i.e., close to the analytical precision of the mass spec technique. No toxic-death were observed among the patients. However, severe toxicities were registered and were associated with overexposure upon the first administration, i.e., when Busulfan was administered following a standard dosing.

Conclusion

Adaptive dosing of HD helps to avoid toxic-deaths by smothering the variability on the global exposure levels. Upfront adaptive dosing (i.e., starting as soon as the first course) could further help to improve the safety of this regimen.

Keywords :
High-Dose Busulfan, pharmacokinetics variability, safety, adaptive dosing

Evaluation of targeted nano-architectures in alternative in vivo models of Head and Neck Squamous Cell Carcinoma

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Cisplatin-based therapies are one of the principal treatments for Head and Neck Squamous Cell Carcinoma (HNSCCs), although associated with systemic toxicities and a low overall patients survival rate. The encapsulation of chemotherapeutics in nanoparticles decorated with targeting agents is a strategy of special interest to improve the chemotherapeutic efficacy while reducing the associated side effects. However, in physiological conditions, serum proteins are adsorbed on the surface of nanomaterials causing protein corona formation, which enables the recognition by phagocytic cells and leads to nanomaterial sequestration.

In this work, a transferrin targeting strategy based on a protein corona-modulating peptide (Tf2) has been integrated with non-persistent nano-architectures (NAs) loaded with a cisplatin prodrug (NAs-cisPt-Tf2). NAs are biodegradable ultrasmall-in-nano materials designed to provide advantageous medical effects as well as to avoid noble metal persistence after their biological action. The therapeutic evaluation of cisplatin alone and loaded in NAs has been performed in both 2D and 3D in vitro system of an HPV-negative cell line HNSCCs (SCC-25), and further confirmed on optimized chorioallantoic membrane (CAM) in vivo tumor models. Beyond a significant reducing effect on the tumor volume, the treatment with NAs-cisPt-Tf2 induced interesting anti-angiogenic and pro-apoptotic effects. The conventional clinical use of cisplatin in treating oral cancer patients and the documented overexpression of the transferrin receptor in oral tumors further highlight the relevance of NAs-cisPt-Tf2. Overall, NAs-cisPt-Tf2 addresses the demand for a targeted and safe non-invasive treatment for oral cancer, in which the conservation of nearby non-malignant tissue is likewise essential.

Novel approaches to hamper cancer associated fibroblasts tumour support

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Introduction

Despite the cancer research budget continuing to grow, together with our knowledge of the complex interaction between the tumour and the host organism, standard treatments are still inefficient, leading to extremely serious side effects and the development of tumour relapses with difficult treatment due to acquired chemoresistance. Cancer Associated Fibroblasts (CAFs), as the main cell population of the tumour microenvironment (TME), play a determinant role in all stages of tumorigenesis, from the start point, tumour initiation, to the final step, the induction of the pre-metastatic niche settlement, thus it seems evident that novel therapeutic approaches should hamper CAFs support to tumour cells.
We have proven in vitro, in vivo and in patients with advanced solid tumours (conducted in the context of a compassionate study), the efficacy of a protein-based treatment. Here, we present the effect of the same formulation on CAFs modulation.

Material and Methods

Firstly, the development of a model of fibroblast malignification through co-culture and 3D culture systems, using cancer stem cells derived from pancreatic BxPc3 cell line and/or BxPc3 cells grown in monolayer; secondly, CAFs characterization using immunofluorescence and Q-PCR analysis; thirdly, the determination of the efficacy of a proteins-based treatment by proliferation/viability assays; clonogenic analyses and wound-healing evaluation.

Results

Fibroblasts malignification was proved by phenotypical and protein expression changes, and in fact, induced CAFs showed an increased expression of αSMA, FAPα and SDF1 when compared with non-tumour fibroblasts (NOFs). In addition, CAFs promoted tumour cells proliferation, viability and migration rate and interestingly, those tumour supporter parameters were decreased after protein-based treatment.

Conclusions

CAFs “re-education” instead of CAFs eradication could be a novel therapeutic strategy to decrease TME influence in drug uptake, immune evasion, tumour progression and further tumour dispersion.

Keywords:
Cancer Associated Fibroblasts; tumour microenvironment

AXL emerges as a promising biomarker and target for pancreatic cancer

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Introduction

PDAC remains one of the deadliest malignancies with an overall 5-year survival rate of only 10%. New biomarkers for early diagnosis and strategies to fight metastasis spreading are warranted. Although not implicated as an oncogenic driver itself, AXL upregulation has been linked to tumor proliferation, invasion and chemoresistance. In this context, we used warfarin, which blocks Gas6-mediated Axl activation and MYD1-72Fc, a decoy receptor with sub-picomolar affinity to GAS6, in in vitro and in vivo models of PDAC.

Methods

AXL prognostic value was first assessed in publicly available databases and then validated by immunohistochemistry (IHC) in tissue-microarrays (TMAs) of two cohorts of radically-resected and liver-metastatic patients. The impact of warfarin and MYD1-72Fc on inhibition of cell growth and migration was studied with SRB, wound-healing and invasion assays. To evaluate the role of AXL on PDAC growth and liver metastases we used mouse orthotopic models of Firefly-transduced primary human PDAC cultures with differential AXL expression and metastatic behavior.

Results

In PDAC tissues AXL levels are significantly higher than in normal pancreas and across several databases high AXL expression was associated with significantly shorter survival. This prognostic role was confirmed by the IHC performed in TMAs, showing a correlation between high levels of AXL expression and worse clinical outcome. Warfarin and MYD1-72Fc had limited cytotoxic activity but inhibited migration and invasion. The orthotopic models showed in vivo metastatic potential through lymph-nodes and liver, as observed in patients. However, the PDAC1 primary culture, harbouring low levels of AXL-expression, had only limited metastases to the liver, while PDAC3 cells, which have high levels of AXL, created both large primary tumors, and numerous liver metastases. Treatment with MYD1-72Fc reduced the number of metastases, inducing tumor necrosis.

Conclusions

Through -omics and IHC, we identified the tyrosine-kinase receptor AXL as an enriched component in PDAC samples, with a prognostic role. Interestingly, we showed that AXL expression correlated with migration/invasion and metastasis in PDAC primary cultures and can be targeted by agents that act on the GAS6/AXL pathway. Collectively, these findings suggest that AXL could constitute a future biomarker and target to be exploited in PDAC.

Keywords:
AXL; Diagnosis; Pancreatic cancer; biomarker

Enhanced cancer-selectivity and antitumour activity in carcinoma cell lines by transferrin receptor-targeted, apoferritin-encapsulated therapeutic agents

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Targeted delivery of anticancer agents could significantly improve treatment for patients. To date a number of drug delivery systems has been considered, among which protein delivery capsules offer biocompatibility, stability in physiological solvents and recognition by receptors1. By exploiting cancer cells` upregulation of transferrin receotor-1 (TfR-1) expression and apoferritin (AFt) recognition, we sought to enhance tumour-targeting and reduce adverse toxicity. Thus, robust, reproducible protocols have been developed to encapsulate therapeutic and experimental anticancer agents within AFt protein cages. The diffusion or nanoreactor route was optimised for encapsulation of >500 imidazotetrazine and >100 benzothiazole molecules2-5, whereas pH-disassembly, reassembly was optimised for encapsulation of natural product derivative Jerantinine A acetate (JAa; ~120 molecules per AFt cage). Upregulated TfR-1 has been demonstrated in cancer cell lines including mammary carcinoma and glioblastoma multiforme (GBM) cells. We have demonstrated significantly more potent activity and cancer-selectivity of AFt-encapsulated agents compared to naked counterparts; a consequence of enhanced internalisation of AFt in cancer cells, compared to MRC5 fibroblasts. Accordingly, AFt-formulations delivered significantly greater intracellular drug levels to cancer cells, than naked agent treatment alone. Moreover, AFt-TMZ was able to overcome TMZ-resistance conferred by MGMT. In MTT assays AFt-TMZ yielded GI50 values <10 μM against MGMT+ve U373M GBM cells; (TMZ alone GI50 >300 μM). Mechanisms of action and molecular targets of anticancer agents remain consistent following AFt-entrapment – e.g. AFt-encapsulated benzothiazoles induce cytochrome P450 expression; AFt-encapsulated JAa evokes G2/M cell cycle arrest, downregulates Mcl-1 expression and cleaves PARP at lower concentrations that naked JAa. AFt alone has negligible effects on cell growth, viability or protein expression.

In conclusion, we demonstrate that AFt represents a biocompatible delivery vehicle for targeted delivery of anticancer agents, such as imidazotrazine analogues, benzothiazoles and Jerantinines to TfR-1-expressing tumours, including GBM and breast cancers, offering potential to minimise toxicity and enhance activity.

New class of reversible benzylpiperidine-based monoacylglycerol lipase (MAGL) inhibitors displaying anti-proliferative activity in pancreatic ductal adenocarcinoma cells

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Monoacylglycerol lipase (MAGL) is a cytosolic serine hydrolase which hydrolyzes brain 2-arachidonoylglycerol, an endocannabinoid neurotransmitter implicated in different physiological and pathological processes such us cancer. MAGL is overexpressed in cell lines of several human cancers and primary tumors and supports cancer aggressiveness, invasiveness and proliferation. Therefore, MAGL inhibitors could be used as potential anticancer agents. Nowadays, only few reversible and selective MAGL inhibitors have been reported in literature; thus, we aimed to develop a new class of reversible MAGL inhibitors. To synthesize the novel benzylpiperidine MAGL inhibitors we followed a convenient synthetic procedure. Biochemical assays (enzymatic inhibition assays, dilution tests, preincubation experiments and kinetic studies) were carried out by adopting a spectrophotometric method. Molecular modeling studies and molecular dynamics simulations were performed to provide a structure-based rationale for the structure-activity relationships of these inhibitors. Pharmacological investigations in pancreatic ductal adenocarcinoma (PDAC) preclinical models, including sulforhodamine-B assay, immunoassay, combination assay and wound-healing assay, supported the potential applicability of the newly synthesized compounds for the treatment of PDAC. We developed a new class of reversible benzylpiperidine-based MAGL inhibitors, among which the most potent MAGL-inhibitor of this series showed a IC50 value of 2.0 nM. This compound is selective towards other targets of the endocannabinoid system, such as, fatty acid amide hydrolase (FAAH) and α/β hydrolase-6 and -12 (ABHD6 and ABHD12). Pharmacological investigations in pancreatic cancer cells showed that this compound has a notable antiproliferative activity in SUIT-2 immortalized cancer cells and in PDAC2 and PDAC3 primary cell cultures (IC50 values ranging from 7.25 to 12.61 μM), compared to immortalized ductal normal cells HPNE (IC50 > 20 μM). In addition, it significantly induced apoptosis in PDAC cells, notably reduced cell migration and, ultimately, exerted a synergistic interaction when combined with chemotherapeutic agent gemcitabine. The newly synthesized benzylpiperidine-based reversible MAGL inhibitors showed promising anti-cancer properties for the treatment of PDAC, supporting future studies to evaluate their potential use in the clinical setting.

Circulating TGF-Beta and PD-L1 in Pancreatic Cancer

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Background

Pancreatic ductal adenocarcinoma (PDAC) is a highly devastating disease with rising incidence and poor prognosis. The lack of reliable prognostic biomarkers hampers the individual evaluation of the survival and recurrence potential. Methods: Here, we investigate the value of plasma levels of two potential key players in molecular mechanisms underlying PDAC aggressiveness and immune evasion, soluble TGF-beta (sTGF-beta) and sPD-L1, in both metastatic and radically-resected PDAC. To this aim we prospectively enrolled 38 PDAC patients and performed appropriate statistical analyses in order to evaluate their correlation, and role in the prediction of disease relapse/progression, and patients' outcome. Results: Metastatic patients showed lower levels of circulating sTGF-beta and higher levels of sPD-L1 compared to radically-resected patients. Moreover, a decrease in sTGF-beta levels (but not sPD-L1) was significantly associated with disease relapse in radically-resected patients. We also observed lower sTGF-beta at disease progression after first-line chemotherapy in metastatic patients, though this change was not statistically significant. We found a significant correlation between the levels of sTGF-beta and sPD-L1 before first-line chemotherapy. Conclusions: These findings support the possible interaction of TGF-beta and PD-L1 pathways and suggest that sTGF-beta and sPD-L1 might synergize and be new potential blood-based biomarkers.

Keywords:
pancreatic adenocarcinoma; prognostic biomarker; liquid biopsy; sTGF-beta; sPD-L1

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PAMM
POSTERS

POSTER | N°1

Quantitative impact of different patient and disease factors on C-reactive protein synthesis, a prognostic biomarker in advanced non-small cell lung cancer (NSCLC) patients

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Background

C-reactive protein (CRP), a marker of inflammation, positively correlates with poorer prognosis and survival in advanced cancers, such as NSCLC. We aimed to identify and quantify the impact of clinical and patient factors that significantly influence the synthesis rate constant of CRP (Kin) in patients with advanced NSCLC to determine the contribution of different factors on CRP concentrations in this patient group.

Methods

CRP data were obtained from 256 advanced NSCLC patients of the CEPAC-TDM study [1] who received paclitaxel 3-weekly combined with a platinum-based drug. CRP was measured using a validated commercial assay on day 1 of cycles 1, 2, 3; day 2 of cycles 1, 2; and at the end of treatment. Longitudinal CRP concentrations were modelled using NONMEM assuming a steady-state turnover model and a first-order degradation rate constant fixed to a t1/2 of 19 h [2]. Different covariates were tested for potential influence on CRP synthesis using a stepwise covariate model building approach [3].

Results

Baseline inflammatory cytokine interleukin-6 (BLIL6), baseline sum of diameters (BLSD), disease stage, and smoking status significantly influenced Kin. Estimated Kin for a non-smoker with stage IV NSCLC, median BLIL6 (2.57 pg/mL) and BLSD (8.25 cm) was 0.304 mg/L/h. There was a 9-fold and 2-fold change per 90% interval of BLIL6 and BLSD, respectively. Compared to non-smokers, former and current smokers had a 58.7% and 105% higher Kin, respectively. A less advanced disease stage (stage IIIB) decreased Kin by 41.8% compared to stage IV. A current smoker, with disease stage IV, BLIL6 (11.9 pg/mL) and a high tumour load (BLSD: 16.9 cm) had an estimated 61-fold higher Kin and thus CRP concentrations compared to a non-smoker with disease stage IIIB, BLSD (2.2 cm) and BLIL6 (0.4 pg/mL).

Conclusion

This work provides a mathematical model for the quantitative impact of patient- and disease-specific factors influencing CRP in advanced NSCLC patients undergoing palliative first-line platinum-based chemotherapy. We will extend this model to describe the relationship between CRP and treatment response/survival to further explore the predictive and prognostic value of CRP in cancer patients.

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POSTER | N°2

Management of nasopharyngeal cancer in Algeria. A retrospective study

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Introduction

Nasopharyngeal cancer is common in Algeria, its worldwide incidence is geographically dependent (1), the diagnosis is often late and its prognosis is reserved despite its high radiosensitivity.

Patients and methods

Retrospective study conducted from January 2019 to August 2022 in the medical oncology department of the University Hospital of Mostaganem on 49 cases of histologically proven nasopharyngeal cancer.

Results

The average age in our series was 50 years [19-81 years] with 83.7% male, 73.5% had no history individuals,with an average diagnostic delay of 4 months [1-20months]. Cervical adenopathies were palpated in 65.3% of cases, otorhinoneurological triad in 10.2% of cases.It was an undifferentiated carcinoma of the nasopharyngeal type in 93.9% of cases.The tumor was classified T4 in 24.5% of cases, T3 (32.6%), T2 (30.6%), T1 (6.1%), N3 in 18.4% of cases and M1 in 8.2% of cases. Neoadjuvant chemotherapy was administered in 83.7% of cases,the protocol used was PTX (CDDP-Docetaxel-Capecitabine) in the majority of cases with an average number of course of 3,4 [1-9 courses], neoadjuvant chemotherapy complete response rate was 18.4%, stability (22.4%), partial response (42.9%), progression (10.2%). The average creatinine level before the start of cisplatin-based chemotherapy was 9.5 mg/l and 11.5 mg/l after the end of treatment. Of the 40 patients who received neoadjuvant radiotherapy, 77.5% did so exclusively and 22.5% concomitantly with chemotherapy,the mean dose delivered was 62.46 Gy [30-70 Gy]. At the end of the treatment, 88.3% of the patients who received neoadjuvant treatment were in complete remission,but 7% of the tumors recurred locally within a year, the five patients in a metastatic situation underwent palliative chemotherapy such as CDDP- Gemcitabine where we concluded one complete response case, one stability case and 3 cases of disease progressions. Four patients died during the study period.

Conclusion

We note an improvement in the management of these cancers, in particular the scheduling of patients in the various radiotherapy departments, however, more efforts must be made to optimize the management of these cancers.

Keywords:
Nasopharyngeal cancer, Retrospective study

POSTER | N°3

Epidemiological profile of colorectal cancers in a region of western Algeria between 2012 and 2016

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Colorectal cancer (CRC) is the most common cancer in women, after breast cancer, and the first cancer in men, well before lung cancer. It is still diagnosed at a late stage with very heavy treatment, which constitutes a real public health problem. The objective of this work is to evaluate the epidemiological profile of colorectal cancers in Tlemcen province; a region of western Algeria; over a period of 5 years (2012-2016).

A retrospective study of 581 cases of colorectal cancer collected at the service of epidemiology and preventive medicine (Tlemcen Hospital) between January 2012 and December 2016 was performed. Epidemiological data were processed using SPSS version 25 and Microsoft Excel 2010.

The epidemiological profile has shown that colorectal cancer in our region ranks 3rd in both sexes. There were 322 men (55.4%) affected compared to 259 women (44.6%) with a sex ratio of 1.2. A predominance of males is noted in 50-60 age group, while for the female sex, the dominance is between 60-70 years old. The mean age of CRC occurrence was 60±13 years, with an extremity ranging from16 to 90. A significantly higher rate was recorded for rectal cancer (43.7%) followed by sigmoid colon (5.7%). Variable rates were recorded during the 5 years with a peak in 2014 (27.9%).

The regular updating of these data, improvement of lifestyle, as well as the implementation of mass screening for CRC based on immunological tests for human blood in the stool will be essential for a better management of this cancer.

Keywords:
colorectal cancer, epidemiology, Algeria, Tlemcen province

POSTER | N°4

Molecular and pharmacological inhibition of ubiquitin-specific protease 8 in cisplatin-sensitive and resistant endometrioid ovarian carcinoma cells

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Introduction

Deubiquitinases (DUBs) mediate the removal of ubiquitin from diverse proteins participating in the regulation of cell survival, DNA damage repair, apoptosis. Previous studies have shown an association between activation of cell survival pathways and platinum-drug resistance in ovarian carcinoma cell lines. Ubiquitin Specific Protease 8 (USP8) appears to be involved in modulation of cancer cell survival. Thus, the aim of this study was to explore if USP8 could be a target for modulation of cisplatin efficacy.

Material and Methods

In this study, we used ovarian carcinoma cells, including cisplatin-resistant variants with increased survival features to evaluate the efficacy of molecular and pharmacological targeting of USP8 as a strategy to overcome drug resistance. We performed biochemical analysis of USP8 activity in pairs of platinum-sensitive and -resistant cells. Drug sensitivity was assessed by colony forming assay and susceptibility to apoptosis was tested by caspase 3/7 activation and annexin V-binding assays. The effect of drug combinations was analyzed using the Chou-Talalay method.

Results

We found increased USP8 activation and levels in resistant compared to sensitive cells. The silencing of USP8 increased cisplatin sensitivity in cisplatin-resistant cells, a phenomenon associated with enhanced susceptibility to drug-induced apoptosis, linked to FLIPL decrease in IGROV-1/Pt1 cells. When looking for a pharmacological inhibitor of USP8, we observed that the caffeic acid phenethyl ester (CAPE) inhibited USP8, but not proteasomal DUBs in cell-free assays. The combination of CAPE with cisplatin in ovarian cell lines of various histotypes showed a synergistic effect in TOV112D cells and in the cisplatin-resistant IGROV-1/Pt1 variant. In the latter cells, persistent G1 accumulation upon combined treatment associated with p27kip1 protein levels was observed. The synergy was not dependent on apoptosis induction, and appeared to occur in cells with higher USP8 levels. In vivo antitumor activity studies supported the advantage of the combination of CAPE and cisplatin in the subcutaneous model of cisplatin-resistant IGROV-1/Pt1 ovarian carcinoma as well as CAPE activity on intraperitoneal disease.

Conclusions

Taken together, our results support that inhibition of USP8 either by molecular or pharmacological tools may be helpful in combination with cisplatin in the treatment of tumors expressing USP8.

POSTER | N°5

Evaluation of the quality of life of patients with early HER2+ breast cancer located at the University Hospital of Oran (Algeria)

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Introduction

In Algeria, breast cancer is one of the chronic and non-communicable diseases whose economic and financial consequences weigh heavily on the financial capacities of the State through the budgets of public health institutions, on the financial balances of the organizations in charge of health insurance.
According to a French inquiry, the proportion of people with a degraded quality of life varies greatly depending on the cancer location. Indeed, breast cancer is the second cancer for which a degraded quality of physical life was observed in a large number of patients (55.8%).

Objectives

The objective of our study is to assess the quality of life of patients with early localized HER2+ breast cancer through the EORTC QLQ-BR45 questionnaire.

Methods

This is a retrospective and monocentric descriptive study involving 43 patients diagnosed between June 2021 and April 2022 carried out at the department of oncology of the Oran Hospital and University Center.
To assess the quality of life of HER2+ breast cancer patients under chemotherapy and targeted therapy, each patient was reviewed individually as part of a follow-up consultation, with an operating sheet including QOL questionnaires from the EORTC.

Results

QOL research is particularly important because it allows for a more comprehensive assessment and provides better insight into the psychological and physical health of patients, as well as the environment in which they live.
Functional assessment includes assessment of body image, future outlook, sexual functioning and pleasure as well as breast satisfaction. 34% of patients have an body image score of 100, which explains why the majority of patients, including 41.86%, have undergone a mastectomy.
The symptomatic assessment encompasses different symptoms including side effects of systemic therapy, hair loss, arm and breast symptoms. The results of one study showed that the severity of symptoms was a predictor of QOL during the early period after surgery. High symptom scores were most often reported within 6 months of diagnosis.
The scales of targeted therapy include symptoms of endocrine therapy, skin fungus and endocrine sexual symptoms.
All patients have an endocrine therapy symptom score greater than 50.

Keywords:
Quality of life, Breast cancer HER2+, non metastatic

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POSTER | N°6

Investigation of COVID-19 and vaccination at the University of Tlemcen (Algeria)

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The novel coronavirus (SARS-CoV-2) infecting humans, causing COVID-19 with symptoms ranging from the common cold to pneumonia has been responsible for the current pandemic that has spread rapidly at record speed leaving many deaths of different ages and different ethnicities.

In order to stop the propagation of this virus, scientists rushed to create several efficient vaccines against this virus, and despite being marketed in all countries of the world, opinions diverged between supporters and opponents. This prompted us to carry out this survey on vaccination against COVID-19 at the University of Tlemcen (western Algeria), through a questionnaire intended for teachers and students launched by email and social networks in a period of 2 Months, with the aim of exploring the perceptions and opinions of participants on the infection.

The majority of respondents for this study belonged to young people with a female gender, despite the latter, men were the most vaccinated, fortunately most of them also acknowledge the association between prevention and vaccination.

So, does the existence of vaccine for this virus indicate that the pandemic is over? Or will new mutations appear?

Keywords:
COVID-19, SARS-CoV-2, Vaccination, a questionnaire, University of Tlemcen.

POSTER | N°7

Determination of the viral load of high-risk human papillomavirus in cervical cancerous and precancerous lesions in a population of western Algeria

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Cervical cancer, which is closely linked to infection with high-risk human papillomavirus (HPV-HR), is the second cancer of women in Algeria . A correlation between HPV-HR, viral load and lesion severity has been well established.

The objective of this work is to search for the most oncogenic HPVs and to specify their viral loads in a population of western Algeria (Tlemcen). Seventy-six (76) cervical samples were used to determine the viral load of HPV 16, 18, 45, 31 and 33 by the real-time PCR technique.

This technique revealed the presence of 7 samples positive in HR-HPVs with a rate of 9.2%. One HPV 16 in a patient with treated squamous cell carcinoma whose viral load was 9.38x106 copies/106 cells, which is a predictive sign of treatment failure and recurrence. Three HPV16 and one HPV18 whose viral loads varied between 7.33x10 6 and 3.87x10 7 HPV copies/10 6 cells were detected in patients with high-grade lesions. These patients should be referred for biopsy/colposcopy . In addition, HPV16 and HPV45 were detected in 2 immunosuppressed patients with low viral loads (1.26x10 3 and 5.72x10 2 respectively). Viral load monitoring for better management of those patients is recommended.

The determination of the HPV-HR viral load is very important in the therapeutic follow-up and the control of patients with precancerous lesions and immunosuppressed patients. It is more effective than simple virus detection in predicting the severity of cervical injury because a high viral load is associated with a decreased likelihood of clearance of HPV infection.

Keywords:
HPV-HR, viral load, real-time PCR, cervical cancer, screening

POSTER | N°8

Anti-proliferative effect HNF4alpha and PFKFB4 siRNAs on hepatocellular carcinoma

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Earlier diagnosis and advances in treatment strategies have increased the average survival of cancer patients over the last decades. Despite the increased number of new anti-neoplastic agents, there has been no adequate therapy for intricate malignancies such as hepatic cancer. Cancer metabolism is the main building block standing behind cancer promotion and progression even in the presence of a harsh environment. Targeting metabolic pathways, such as glycolysis and metabolic regulators, is regarded as a promising new strategy for cancer treatment. The current study is to investigate the effect of knocking-down hepatic cancer glycolytic and metabolic regulators (PFKFB4, and HNF4-α) respectively, on cell's viability using siRNA each one alone and in combination. The human hepatocellular carcinoma cell line, HepG2, was used to study the anti-proliferative activity of targeting PFKFB4 and HNF4-α mRNAs by transfection with siRNAs, each one alone and in combination. A conformational study was done to investigate the silencing of the above genes on their expression level using RT-PCR. siPFKFB4, and siHNF-4α decreased tumor cell proliferation with a maximum effect shown with combining both siPFKFB4 and siHNF-4; followed by silencing of HNF4-α genes alone. Moreover, the least anti-proliferative effect was observed when silencing PFKFB4 gene alone. Silencing of both genes was confirmed using qPCR technique and our results revealed that both genes were knocked-down upon treatment with siPFKFB4, and siHNF-4 α. This study demonstrated the major tumor promoting and progressive effects of HNF4-α, while PFKFB had modulator effects on the viability of HepG2. Our data also suggest the ability of hepatic cancer cells to find alternative metabolic pathways other than glycolysis to survive. Silencing of both HNF4-α and PFKFB genes together reveals the modest anti-proliferative effect suggesting a more comprehensive effect on targeting several metabolic pathways, making hepatic cells less able to survive.

Keywords:
siRNA, HNF4alpha, PFKFB4, Cancer

POSTER | N°9

Epstein-Barr virus in Algerian Breast Cancer. "Research of the Genome and Quantification of Viral Load and correlation with expression of genes of Matrix metalloproteinase and VEGF"

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Nearly every person is infected with the Epstein-Barr-Virus (EBV) and remains infected for life. While almost all EBV infections are benign, a small percentage of infected persons develop cancers. EBV is associated with 100% of NPC (Undifferentiated Nasopharyngeal Carcinoma). This type of cancer is increasing in Algeria with other forms of cancer also supposed to be linked to EBV infections in our country. Recent studies suggested a link between EBV and breast cancer which can provide new insights and help identify women at risk using the virus as a tumor marker. The association of EBV with breast cancer remains controversial. A study on the presence of EBV and the quantification of the viral load in breast tumors (frozen biopsies) was realized using quantitative real time PCR. These results show that the EBV same tumor. EBV genome is heterogeneously distributed in the tumor and this could be due to either a poor storage of samples, the heterogeneity of cancerous tissues or even genome was detected in approximately 78% of tumor samples and in different DNA extractions from several pieces within the same tumor; however the number of copies of EBV remains low. The viral load was found to be highly variable from one tumor to another and within the to the limit of sensitivity of the technique used. We also investigated the expression of a panel of genes implicated in angiogenesis (VEGF) and matrix metalloproteinase (MMPs) in our samples. The aim of this study is to explore these genes and correlate their expression with the tumors that are EBV-positive, using quantitative reverse transcriptase (RT-PCR). We focused on MMP2, MMP9, MMP14, EMMPRIN, VEGF165, VEGF121, we examined whether the RNA expressions of these MMPs and VEGF are associated with high viral load. The results obtained clearly indicate that the product of some of these genes is indeed present in our samples, and their quantity is greater in EBV positive tumors compared to EBV negative cells. All of these results allow us to suggest that EBV positive samples could have a role in tumor development and progression; in fact EBV infected cells could secrete factors involved in tumor invasion such as MMPs and VEGF angiogenesis. Positive correlation with tumor EBV+ and VEGF and MMP genes.

POSTER | N°10

Unraveling the polypharmacology of beta-blockers in neuroblastoma using chemoproteomics

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Introduction

Our lab has previously shown that anti-hypertensive drugs, β-blockers, can increase the efficacy of chemotherapy in several cancers including neuroblastoma. The mechanism(s) involved remains however poorly understood. First, we showed that the enantiomers of propranolol and carvedilol (R-S) were equipotent at increasing the cytotoxic activity of vincristine in neuroblastoma cells. Since the (R)-enantiomers have low affinity for the β-adrenergic receptors, we concluded that their canonical targets are not involved in this effect. Therefore, this project aims at developing an innovative approach to identify the non-canonical targets of beta-blockers and thus to better understand their mechanism of action.

Methods

We developed an integrative method that combines unsupervised and candidate strategies. Using two complementary and independent chemoproteomics approaches, our first goal was to uncover the interactome of β-blockers in neuroblastoma. Based on our results, we then performed pharmaco-metabolomics analyses.

Results

We used click chemistry-based proteomics, a biocompatible chemical reaction coupled with mass spectrometry, and found an enrichment in proteins involved in cell metabolism within the 77 identified interactors shared by the three tested β-blockers. In parallel, we exploited a biophysical assay called “cellular thermal shift assay” coupled with quantitative mass spectrometry (MS-CETSA) to evaluate the impact of beta-blockers (propranolol and carvedilol) and vincristine alone and in combination in cellulo. Our results highlighted well-known targets of vincristine such as tubulins, but also an enrichment in proteins involved in cell metabolism and mitochondrial respiration in both monotherapies and combination treatment. We then performed 13C glucose and 13C glutamine tracer experiments and showed an alteration of the glucosamine and pyrimidine synthesis pathways under the combination treatment. By using functional genomics and coupling our clickable drug derivatives with an azide-fluorophore to realize co-localization experiments, we will next validate and characterize the identified metabolic targets of β-blockers impacting neuroblastoma biology and drug response. Overall, our results show that β-blockers increase the efficacy of chemotherapy agents in neuroblastoma by interfering with cancer cell metabolism, independently of beta-adrenergic receptors.

Conclusion

This project, which uses already-approved drugs to discover new therapeutic targets by uncovering their poly-pharmacology, could open major avenues for the development of biology-guided drug repurposing strategies.

Keywords:
Pharmacology, oncology, proteomics

POSTER | N°11

Prescreening of potential germline pharmacogenotypes during the process of tumors sequencing programs

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Introduction-objectives

Tumor molecular panels routinely identify oncogenic drivers, allowing individualized cancer therapy.

Due to somatic-and-germline-variant tumor DNA composition, tumor DNA sequencing might reveal germline information. This study aimed to assess the feasibility and the relevance of identifying potential pharmacogenotypes on tumor sequencing.

Methods

Five pharmacogenes (TPMT, NUDT15, DPYD, CYP2D6, UGT1A1) were added to a large cancer-gene panel (CGP) that targets all exonic regions of 108 genes. The library was prepared with SureSelectXT-HS(Agilent) and sequenced with NextSeq550DX(Illumina).

Seventy-two tumoral samples –including five ones with a significant UGT1A1(2/5) or DPYD(3/5) germline pharmacogenotypes- underwent the CGP screening for theranostic purpose.

Results

In this 72-solid-cancer-patients cohort, most patients presented with ovarian(n=22), breast(n=17) or colorectal(n=7) cancer. Three samples were uninterpretable due to insufficient DNA quality.

All five control tumoral samples matched with the external germline pharmacogenotypes. Fifty-four patients presented unknown pharmacogenetic variant. Fifteen seemed to have at least one theoretical actionable variant. Six patients presented more than one likely-actionable variant, including four ones with a closer-clinical-monitoring-requiring variant.

One DPYD variant (c.1236G>A/HapB3) in two patients, three TPMT variants in seven patients (TPMT*2(n=1), TPMT*3A(n=4), TPMT*3C(n=2)), one NUDT15*3(c.415C>T) in two patients and one UGT1A1*27(c.686C>A) variant have been found with a near-to-50% variant-allele-fraction. The UGT1A1*28(c.-53_-52insTA) has been detected in twenty-eight patients: twenty-one heterozygous-like and seven homozygous-like UGT1A1*28.

In total, one potential DPYD genotype and two potential homozygous-like UGT1A1*28 seemed to be clinically actionable and five potential heterozygous UGT1A1*28 variant would indicate a closer therapeutic monitoring.

Although tumoral instability -and particularly loss-of-heterozygosity- can misrepresent CYP2D6 copy numbers variations, seventeen CYP2D6 metabolization profiles seemed clinically actionable for cancer or supportive treatment, out of the thirty-four relevant CYP2D6 diplotypes.

Out of the seventy-two samples were suspected six potential ultrarapid metabolizers, eleven poor metabolizers, eighteen intermediate metabolizers and twenty-five normal metabolizers. Twelve were uninterpretable due to insufficient variant depth (n=7) or undetermined impact (n=5).

Conclusion

Pharmacogenes variants can be correctly identified on tumor samples. By revealing potential presence of likely-pharmacologically-actionable pharmacogenetics variants, tumor sequencing can propose a targeted germline pharmacogenetics test. Integration of pharmacogenetics in the tumor worflow analysis will certainly need clinical prospective study to confirm its useness.

Keywords :
Pharmacogenetics, Tumor sequencing, DPYD, UGT1A1, CYP2D6

POSTER | N°12

A new therapy against Cancer Stem Cells using polystyrene conjugated nanoparticles

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Introduction

According to the World Health Organization cancer is the second leading cause of death globally. Although in the last years successful oncological therapies have been developed metastasis and tumor recurrence still have a high death rate impact on advanced cancers patients. Cancer Stem Cells (CSCs) have been described as a subpopulation of tumor cells, with similar characteristics of stem cells, that drive metastasis and can cause tumour relapse. Irregular expression of several miRNAs has been associated with CSCs. Particularly, miRNA-21 has been proved to regulate cell apoptosis. Here we have proven the effect of anti-miRNAs polystyrene nanoparticles on CSCs derived from melanoma as a possible novel antitumoral therapy.

Material and methods

Anti-miRNAs sequences were conjugated to polystyrene nanoparticles and tested on the malignant melanoma cell line (Mel1). Different types of nanoparticles were synthesized i) loaded with a fluorophore; ii) loaded with antimiRNA21 and iii) loaded with both anti-miRNA21 and a fluorophore. 3D culture was used for the formation of cancer stem cell spheres.

Results

24 hours post-treatment 50% of Mel1 derived CSCs showed uptake of antimiRNAs Polyestyrene nanoparticles as shown by microscopy images. Further, the effect of the anti-miRNA-loaded nanoparticles was demonstrated on CSCs by apoptosis assays with an increased number of apoptotic cells compared with control after 3 days of nanoinfection.

Conclusion

The use of nanoparticles conjugated with anti-miRNA can represent a new strategy against CSCs.

POSTER | N°13

Sirolimus activity in patient-derived epitheliod hemagioendothelioma models: the role of GDF-15

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Introduction

Epithelioid hemangioendothelioma (EHE) is an ultra-rare soft-tissue sarcoma with heterogeneous presentation and clinical behavior. Approximately 90% of EHE cases harbor the WWTR1-CAMTA1 translocation, leading to Hippo-pathway activation. There is no standard treatment for EHE and patients with aggressive disease have a poor prognosis, highlighting the need for novel effective treatments. Considering the important interplay between the Hippo and PI(3)K–Akt–mTOR pathways, in this study we pre-clinically assessed the activity of sirolimus in a patient-derived xenograft (PDX) and the corresponding cell line that we developed and currently represent the only human models of the disease available worldwide.

Materials and methods

A PDX was established from a patient affected by high-grade EHE and characterized for consistency with the originating clinical tumor by histology, FISH and RNA-sequencing. We comparatively evaluated the activity of sirolimus and doxorubicin in the PDX model by assessing tumor volume inhibition (TVI). In EHE cells, drug cytotoxic activity and apoptosis were evaluated by SRB and TUNEL assay, respectively. The expression of mTOR downstream proteins was assessed by western blotting. Expression of inflammatory cytokines was detected by ELISA in cell culture media and mouse blood.

Results

In the EHE PDX model, sirolimus showed a significant antitumor activity (max TVI 80%) compared to doxorubicin, which almost negligibly affected tumor growth. Consistently, sirolimus was more active than doxorubicin also in EHE cells (IC50 value: 0.01 vs 0.1 μM) and induced a greater apoptotic response (TUNEL-positive cells: 20 vs 10%). Moreover, sirolimus down-regulated the mTOR pathway both in vitro and in vivo. Interestingly, sirolimus reduced the release of growth & differentiation factor-15 (GDF-15, an inflammatory cytokine whose expression levels were found significantly higher in blood of patients with aggressive EHE compared to those with a more indolent disease) from EHE cells, as detected in cell culture medium and mouse blood. Preliminary evidence also indicated that siRNA-mediated knockdown of GDF-15 in EHE cells enhance their sensitivity to sirolimus.

Conclusions

Our results indicate sirolimus as a promising drug for the treatment of EHE patients and suggest a possible role for the GDF-15 as a biomarker to monitor the disease response to sirolimus treatment.

POSTER | N°14

Rubinib: a multitarget drug with a potent in vitro and in vivo effect against CSCs

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Introduction

Currently the existence of an intrinsic heterogeneity among the tumor cells is explained by the presence of several cell types, each one fulfilling a specific function. In this context, a minority of the tumor cell population (1-5%) called cancer stem cells (CSCs) are responsible of tumor initiation, metastasis, resistance and relapses in patient that previously responded to treatment [1]. Due to their important role, CSCs have become one of the main focuses in the search of new targeting drugs capable of attacking these aggressive subpopulations [2]. In this work we have designed, obtained and synthetized a novel small compound named Rubinib and have studied its ability to target CSCs in several tumor types.

Material and methods

We did cytotoxic assays to demonstrate the selectivity of Rubinib against CSCs isolated from several tumor cells types (breast colon, pancreas and melanoma). Also, we determined the efficacy to decrease stemness properties including among others inhibition of spheres size and number, clonogenicity and the analysis by flow cytometry of surface markers, ALDH1 activity, side population, cell cycle and apoptosis. In addition, microarray analysis was done to determine the mechanism of action of Rubinib. Finally, ADME-tox and in vivo anti-CSCs efficacy were performed.

Results

Rubinib showed an IC50 in the nanomolar range on CSCs subpopulations, inhibiting spheres formation, reducing side population and different characteristic CSCs markers, also promoting cell cycle arrest and apoptosis induction. Data obtained from genomic and proteomic analyses, suggested interesting molecular targets. Rubinib not only inhibited tumorigensis but also was able to inhibit tumor development without any toxicity in mice, showing great efficacy on PDXs from pancreatic cancer. In addition, positive ADME-Tox results were obtained indicating that Rubinib has great bioavailability.

Conclusions

Preclinical results obtained in this work indicate that Rubinib is a very attractive anti-CSCs agent that opens a new strategy in cancer chemotherapy with great potential to promote a clinical trial in patients

Keywords:
Cancer stem cells, Rubinib, PDX, ADME-Tox, tumorigenesis

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POSTER | N°15

The effect of lactate dehydrogenase A and glucose transporters inhibitors on malignant mesothelioma and no-cancer cells' metabolism

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Introduction

Lactate dehydrogenase-A (LDH-A) and glucose transporter type-I (GLUT-1) are two of the key proteins driving glycolysis, a process typically exploited by cancer cells to produce energy. High expression of LDH-A is observed, among other tumors, in aggressive and resistant malignant mesothelioma (MM). We evaluated the cytotoxicity, influence at nucleotide pools, respiration and glycolysis of the LDH-A and GLUT-1 inhibitors at MM and non-cancer cell lines.

Methods

The 50% inhibitory concentrations of cell growth (IC50) were tested with the sulforhodamine B (SRB) assay after 72hrs treatment with LDH-A (PIFLY124) and GLUT-1 (PGL14) inhibitors in a range of 0.1 – 60μM in MM cells (pleural cell lines: H28, H2452; primary peritoneal cultures: MESO-II, STO) and non-cancer cells (HMEC-1). The intracellular nucleotide concentration was measured using RP-HPLC, mitochondrial respiration and glycolysis were analyzed using Agilent Seahorse Analyzer, using the inhibitors (alone or in combination) at IC50 concentrations for 24hrs.

Results

Both compounds showed considerable growth inhibition with the lowest IC50 values for the GLUT-1 and LDH-A inhibitor ranging from: 6.4 to 18.5μM in MM cells, while PIFLY_124 IC50 in the HMEC-1 was 50μM. The combination treatment led to 80% growth inhibition in all the MM models. In the H2052 cell, the ATP levels decreased from 37 to 21 and 8, NAD from 7 to 2 and 1 (nmol/mg protein), and NADH/NAD ratio increased from 0.1 to 0.3 and 0.5 (after PGL14 and PIFLY124 treatment, respectively). The decrease in nucleotide pool was more considerable in pleural than peritoneal mesotheliomas, and combination treatment led to a more significant lowering than control and single treatments. In the HMEC-1 cells ATP reduction was 10% less compared to the decrease in the H2452 line at a similar concentration of inhibitors. The inhibitors had a minor influence on mitochondrial respiration in MM cells.

Conclusion

GLUT-1 and LDH-A inhibitors showed cytotoxicity to mesothelioma cell lines and their combination resulted in a synergistic effect with only slight changes occurring in the non-cancer cell. Our results support further studies to develop these compounds as a potential new therapeutic strategy for the treatment of MM.

POSTER | N°16

Interferon-alpha decreases cancer stem cell properties and synergizes with Vemurafenib in Malignant Melanoma

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Malignant Melanoma (MM) disseminates to distant organs and shows resistance, in part due to melanoma cancer stem cells (CSCs) subpopulations. Non metastatic high-risk melanoma is still treated with a controversial high dose of interferon-α (IFN-α) with a significant improvement in disease free survival in patients, presenting anti-proliferative, anti-angiogenic and immune-modulator properties, however its specific activity over melanoma is still unknown. In this study, we have analyzed the effect of IFN-α-treatment alone or in combination with Vemurafenib drug over melanospheres enriched in CSCs subpopulations studying the ALDH activity, the side population, and specific surface markers exppression by flow cytometry. Our results showed that IFN-α decreased the melanospheres formation and stemness properties including a significant reduction in the ability to form tumors in mice xenotransplants and an interesting exosomes modulation. In addition, the antitumor effect of Interferon has been considerably enhanced during combination with different doses of Vemurafenib. Since new immunotherapies and BRAF/MEK-directed targeted therapies are being imposed in melanoma, as well as different combinations are under clinical trial, the efficacy of interferons over CSCs even at low doses with fewer side effects, should be considered as a potentially combination treatment against the relapse of the disease in oncology

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POSTER | N°17

Differential sensitivity to ionizing radiation in gemcitabine- and paclitaxel-resistant pancreatic cancer cells

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Introduction

Chemoresistance remains a major challenge in the treatment of pancreatic ductal adenocarcinoma (PDAC). Chemoradiation is effective in other types of solid tumors (e.g. glioblastoma), however, sensitivity to ionizing radiation (IR) upon acquired chemoresistance remains unexplored in PDAC. In this study, we investigated the sensitivity of gemcitabine- and paclitaxel-resistant PDAC cells to IR and performed omics and functional studies to unravel the underlying mechanisms.

Materials and Methods

Gemcitabine-resistant (GR) and paclitaxel-resistant (PR) clones were generated from two PDAC cell lines, PATU-T and SUIT2-007 and characterized for their proteomics profiles. Lentiviral transduction was employed to assess cell viability of 3D spheroids through stable expression of firefly luciferase. Spheroids were formed and followed over time after IR exposure. Bioluminescence signal was determined as a measure of cell viability. Cell survival in 2D monolayer was evaluated using colony formation assay. IR-induced DNA damage, apoptosis and autophagy were evaluated using Western blot, qPCR, extra-long PCR and immunofluorescence.

Results

PATU-T PR cells showed increased sensitivity to radiation, while PATU-T GR and wild-type (WT) cells demonstrated similar sensitivity in both 2D and 3D spheroids. IR exposure at 4 Gy inhibited cell growth by 70% compared to 55% in PATU-T GR and PATU-T WT 10 days post-radiation treatment. Proteomics data identified MST4, a radioresistant gene, as significantly downregulated in PATU-T PR cells. We investigated the MST4-ATGB4 autophagy pathway by knockdown in WT and GR cells to validate its radiosensitizing effect.

Conclusion

PATU-T PR cells were more sensitive to IR in both monolayer and spheroid cultures, suggesting that chemoresistance could be overcome by IR. In addition, new insights on molecular pathways underlying this mechanism might provide novel therapeutic strategies.

POSTER | N°18

Prognostic significance of integrin alpha 2 (ITGA2) and role of stiffness in resistance to gemcitabine in pancreatic ductal adenocarcinoma (PDAC)

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive and chemoresistant cancer, with a poor overall survival. The stiff stroma surrounding PDAC is involved in chemoresistance via mechanical cues, although the mechanisms behind it are poorly understood. In this study, biomechanical and pharmacological approaches investigated the cell-adhesion molecule integrin alpha 2 (ITGA2), a cellular mechanical sensor, for its involvement in gemcitabine resistance in PDAC.

Methods

ITGA2 prognostic value was first assessed in GEPIA database, then validated by qPCR and immunohistochemistry in two cohorts of radically-resected and metastatic patients receiving gemcitabine monotherapy. RNA-sequencing and proteomics were used to investigate parental cell line PANC-1 and its gemcitabine resistant clone PANC-1R. The role of stiffness and ITGA2 in migration, chemoresistance and apoptosis was assessed by use of hydrogel-coated wells with tunable stiffness. Spheroids embedding in collagen investigated matrix invasion and remodeling, while siRNA-mediated knockdown assessed restored chemosensitivity.

Results

Patients with high-expression of ITGA2 had a significantly shorter overall survival and progression free survival, suggesting its involvement in gemcitabine resistance. This finding was corroborated in in vitro experiments, where ITGA2 was found overexpressed in gemcitabine resistant cells PANC-1R. Next, by silencing ITGA2 a significant reduction of migratory potential and drug resistance was observed. Moreover, PDAC cells sensitive to gemcitabine showed an increased ITGA2 expression and drug resistance when growing on substrates resembling PDAC stiffness. Finally, gemcitabine resistant cells PANC-1R embedded in soft/stiff collagen showed a significant matrix remodeling and spreading potential via increased expression of CXCR4 and MMP2.

Conclusion

ITGA2 emerged as a novel prognostic factor and its modulation in PDAC cells highlights the relevance of stroma mechanical properties as new potential therapeutic targets to counteract gemcitabine resistance in PDAC.

POSTER | N°19

Potential role of SF3B1 as prognostic factor and new therapeutic target in pancreatic cancer

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Introduction

Current therapeutic strategies to treat pancreatic ductal adenocarcinoma (PDAC) patients fail to deliver successful cures and the survival rates remain dramatically low. Recent studies suggested that modulators of the key splicing factor SF3B1 proved highly efficient against a range of solid and hematological malignancies. However, they have never been tested in PDAC.

In this study, we evaluated the antitumor effects of the SF3B1 modulator "E7107" in primary PDAC cells. Moreover, we analysed the expression levels of SF3B1 in patient tissues and established its association with clinical outcomes.

Materials and methods

We performed a transcriptome-wide characterization of splicing profiles in 5 PDAC primary cell cultures compared to 2 immortalized normal ductal epithelial cell lines to identify differentially spliced genes. The antiproliferative activity of E7107 was assessed by sulforhodamine-B (SRB) assay, while its effect on cell migration and RON splicing was evaluated by wound-healing assay and PCR. Lastly, SF3B1 expression was evaluated in tissue microarrays (TMAs), including paraffin-embedded PDAC samples from 87 patients.

Results

RNA-sequencing based differential splicing analysis revealed a total of 420 significant differential splicing events affecting 340 genes largely involved in the regulation of mRNA splicing, gene expression, and nucleic acid metabolism. Primary PDAC cells displayed a high sensitivity toward SF3B1 modulation by E7107, with 50% inhibitory concentration of cell growth (IC50) values in the low nanomolar range (IC50: 0.16 nM and 0.58 nM in PDAC5 and PDAC3 cells, respectively). Moreover, this compound significantly reduced cellular migration, associated with splicing alteration of RON. Finally, SF3B1 expression was significantly correlated with overall survival and progression-free survival with a hazard ratio of 1.791 (95%CI: 1.144-2.803; p=0.011) and 1.770 (1.141 - 2.745; p=0.012), respectively.

Conclusion

This study supports the rationale that drugs modulating the spliceosomal activity could constitute an attractive therapeutic option for PDAC patients. Moreover, our results assign a new prognostic role to SF3B1, which prompt validation studies and may be extended to other spliceosome components.

POSTER | N°20

The long non-coding RNA COMETT as new potential target in BRAFV600E-mutated papillary and anaplastic thyroid carcinomas

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Introduction

The deregulation of long non-coding RNAs (lncRNAs) expression has been reported in several tumor types [1], including thyroid cancer, and their targeting is emerging as new potential therapeutic strategy [2-3]. Interestingly, we recently identified COMETT as an oncogenic lncRNA highly expressed in papillary thyroid carcinomas (PTCs) with BRAFV600E mutation and RET rearrangements, and we reported that its siRNA-mediated silencing impairs oncogenic properties of RET-rearranged PTC cells [4]. Hence, we investigated the oncogenic properties of COMETT in BRAFV600E mutated cells (B-CPAP) and explored its silencing as adjuvant to standard therapy based on vemurafenib, a specific BRAFV600E inhibitor.

Methods

Antisense LNA-modified GapmeRs were used to specifically and potently knockdown (KD) COMETT in two BRAF-mutated cell lines (B-CPAP for PTC and 8505c for ATC). RNA-Sequencing, cell viability and colony forming assays were performed in COMETT-KD cells. COMETT RNA pull-down followed by mass spectrometry (MS) analysis in B-CPAP cells were used to identify COMETT binding partners. Cell viability assay was also evaluated in COMETT-KD BRAF-mutated cells treated (or not) with different vemurafenib doses. Genome engineering (CRISPR/dCas9 system) was used to generate B-CPAP clones stably over-expressing COMETT lncRNA.

Results

Transcriptome analysis of COMETT-KD B-CPAP cells indicated a marked repression of EGFR, WNT and JAK-STAT signaling and of DNA replication and cell cycle processes, paralleled by the increase of pro-inflammatory and apoptotic genes. Preliminary MS data analysis reveals the binding of COMETT to splicing factors (SR and hnRNP) and multiple ribosomal proteins. Interestingly, COMETT KD markedly reduced viability and colony forming ability of PTC and ATC cells, also enhancing their responsiveness to B-raf inhibition in terms of reduced cell viability, even at low drug doses. Functional assays in CRISPR/dCas9 engineered tumor cells to clarify its oncogenic properties are in progress.

Conclusions

Overall, we highlighted the oncogenic role of COMETT in BRAF-mutated PTC and ATC. Moreover, our results suggest that its silencing by ASO Gapmer technology may open new perspective towards adjuvant strategies to overcome drug resistance in BRAF-mutated thyroid tumors.

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POSTER | N°21

Synthesis and biological evaluation of new imidazo[2,1-b][1,3,4]thiadiazole derivates on PDAC cells

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is among the most lethal human cancers, showing only 10% of 5-year survival rate. Early metastasis and chemoresistance are two hallmarks of PDAC, which make the identification of new therapeutic strategies very urgent. This study focuses on the identification of new antitumor compounds targeting aberrant tyrosine kinases with cytotoxic and anti-metastatic activity against pancreatic diseases.

Methods

A new series of imidazo[2,1-b][1,3,4]thiadiazole derivatives has been successfully synthesized and evaluated against PDAC cell lines (BxPC-3, Capan-1, PANC-1, Patu-T, Suit-2), including a gemcitabine-resistant clone (PANC-1-GR) and a primary culture (PDAC-3). With Sulforhodamine B (SRB) assay at 3 concentrations (0.1,1,10μM) we screened twenty compounds and selected compounds which showed the most potent antiproliferative activity for further assays to evaluate their ability to inhibit migration, and induce apoptosis by wound-healing and cytometry assays. Besides, the most potent compound was evaluated on PamGene tyrosine kinase peptide substrate array (PamChip).

Results

Among the new synthesized imidazothiadiazoles, four compounds showed interesting antiproliferative activity against seven PDAC in vitro models (including a primary cell culture) showing IC50 values in the micromolar and low micromolar range. Of note, the IC50s of these compounds were similar in the PANC-1 and PANC-1-GR cells. Additionally, two of them determined a significant decrease of cells migration rate. High-throughput arrays revealed a significant inhibition of the phosphorylation of 49 tyrosine kinases substrates, highlighting Focal Adhesion Kinases (FAK) as an important hub.

Conclusion

New series of imidazo[2,1-b][1,3,4]thiadiazoles have been successfully synthetized and evaluated on PDAC cells. Four novel imidazothiadiazoles showed encouraging antiproliferative aciti-vity, whereas two selected derivates displayed potent antimigratory effects. Cytotoxicity activity and migration decrease could be connected with the imidazothiadiazole ability to inhibit several protein tyrosine kinases, in particular FAK which has a key role in tumor growth, progression and metastasis. In summary, our study confirmed and expanded the value of the imidazo[2,1-b][1,3,4]thiadiazole scaffold in the development of efficacious anticancer therapies.

POSTER | N°22

Epidemiology of Gynecologic cancers in Oran (western Algeria)

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Background

Gynecologic Cancers (cervical, ovarian, uterine, vaginal, and vulvar).are the most common cancers in women and hence an important public health issue. In 2020, Cervical Cancer (CC) is ranking as the fourth with an estimated 604.127 new cases and Ovarian Cancer (OC) is the seventh most common for females with nearly 313.959 new cases worldwide. The frequency of this cancer and its consequences has been declining for 40 years in developed countries thanks to smear Pap. In Oran (westen Algeria, CC is also the second most common cancer among females with an incidence of 12,8 /100000, Uterine cancer is in the eighth place with 4,4/100000 OC is ranking as the sixth with 3,8 cases /100000 and (Oran cancer registry data 2017)

Aim

Describe epidemiological profile of gynecologic cancer in the Oran university hospital (EHUO) .

Methods

The data were obtained from two years of registration (2016-2018) as part of the cancer registry of EHUO. The central coding is carried out using the supports of CIMO2 and CIM10
Results were obtain with Epida analysis

Results

Gynecological cancers. In total, 239 cases of gynecological cancers were resgistered. It represent 16% of all cancers in women. The average age of patients is 56.8±2.0 years old. Regarding the anatomical locations, cervical cancer remains the most common 67.1% followed by that of ovaries 17.5%, followed respectively by uterine, vulvar cancers. Histological analysis of cervical cancerous cases showed a predominance of squamous cell carcinomas 66.9%, Low grades lesions I and II are the most frequent 91.2%.

Conclusion

A good knowledge of the epidemiology of gynecologic cancer will make it possible to put in place measures of prevention, control and treatment, which will contribute to the better health of women.

Keywords:
gynecologic cancer, Epidemiology, registry

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POSTER | N°23

Renal impairment and DPD testing: Watch out for false-positive results!

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Introduction

Measuring uracil (U) levels in plasma is a convenient surrogate to establish dihydropyrimidine dehydrogenase (DPD) status in patients scheduled with 5-fluorouracil (5-FU) or capecitabine. Since this status can translate into adaptive dosing to secure the administration, the influence of renal impairment on U levels and to what extent it could be a confounding factor is a rising concern.

Methods

Here, we report the case of a cancer patient with severe renal impairment scheduled for 5-FU-based regimen. Determination of his DPD status was complicated because of his condition and the influence of intermittent haemodialysis when monitoring U levels. The patient was initially identified as markedly DPD-deficient upon U measurement (i.e., U = 40 ng/mL), but further monitoring between and immediately after dialysis showed mild deficiency only (i.e., U = 34 and U = 19 ng/mL, respectively). Despite this discrepancy, a starting dose of 5-FU was cut by 50% upon treatment initiation. Tolerance was good and 5-FU dosing was next shifted to 25% reduction, then further shifted to normal dosing at the 5th course, with still no sign for drug-related toxicities.

Results

It appears that the first sample was drawn before his dialysis session, while the following ones were taken between two dialysis sessions, and after. Despite being always above the threshold associated with DPD deficiency (i.e., >16 ng/mL), the marked changes in U concentrations throughout time indicate that timing of the sampling with respect to dialysis sessions has a strong impact on measured U levels in plasma and leads to erratic values. Furthermore, DPYD genotyping showed none of the four allelic variants usually associated with loss of DPD activity. Of note, the excellent tolerance upon standard dosing strongly suggests that this patient was actually not DPD-deficient, despite U values always above normal concentrations.

Conclusion

This case report highlights how critical is the information regarding the renal function of patients with cancer when phenotyping DPD using U plasma as a surrogate, and that U accumulation in patients with such condition is likely to yield false-positive results.

Keywords:
5-FU, dialysis, DPD testing, renal impairment, safety

POSTER | N°24

Suicide Gene Therapy Against Cancer Stem Cells

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Introduction

Cancer is one of the main causes of death worldwide, only surpassed by some cardiovascular diseases. Focusing on women, breast and cervical cancer are in the ranking of most common cancer. In 2018, the number of cases was 2.1 million cases of breast cancer and almost 0.6 million cases of cervical cancer. The complexities that underlie cancer reside in mechanisms causing the development of the tumor, its staging, diagnostic, prognostic and difficult treatment. Most known treatments are surgery, chemotherapy and radiotherapy. However, in the majority of the cases these treatments are not as effective as it is wanted, being able to cause the relapse in the patient. Moreover, in cervical cancer, the surgically remove of the uterus causes the infertility of the patient, which allows to have an idea of the aggressiveness of these therapies. Therefore, researches in new therapies against cancer, more effective and less aggressive, are expanding. Currently, one of the most interesting therapies is suicide gene therapy. In the direct way, it consists in the introduction of a gene responsible of the production of a toxin, which will destroy the cell from his inside. In the present manuscript our purpose was to investigate the antitumor efficacy of ldrB gene under the control of different promoters (induced or tumor-specific promoter) in cervix (HeLa) and breast (MCF-7) cancer cells.

Methods

Experiments were conducted in vitro in 2D and 3D culture model and in vivo using NOD SCID mice.

Results

Our results showed that ldrB gene expression under the control of tumor-specific promoter causes a drastic inhibition of HeLa and MCF-7 cells proliferation in vitro in both 2D and 3D models. Moreover, an important decrease on cell viability was observed by ATPlite analysis. Furthermore, ldrB gene induced a severe loss of proliferation in vivo without any side effects in our animal model.

Conclusion

Taking into account our results, this combination of gene and promoter could be a great option in future breast and cervical cancer therapies.

Keywords:
Genes therapy; suicide genes; ldrB; specific tissue promoter; breast cancer; cervical cancer; cancer stem cells.

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POSTER | N°25

Differential miRNA expression of hypoxic MCF7 and PANC-1 cells

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Background
Hypoxia plays a critical role in the tumor microenvironment by affecting cellular proliferation, metabolism, apoptosis, DNA repair, and chemoresistance. Since hypoxia provokes a distinct shift of microRNA, it will be important to illustrate the relative contribution of each hypoxamiRs to cancer progression.

Aims
The present study aims to shed light on the hypoxamiRs that are involved in pancreatic and breast cancer progression to highlight novel targets for new therapies development.

Methods
For 20 cycles, MCF7 breast cancer cells and PANC-1 pancreatic cancer cells were subjected to chronic cyclic hypoxia, which consisted of 72 hours of hypoxia followed by 24 hours of reoxygenation. After 10 and 20 cycles of hypoxia, miRNA expression alterations were profiled using RT-PCR array and further analyzed using a visual analytics platform. The MTT cell proliferation assay was used to determine hypoxic cells' chemoresistance to doxorubicin.

Results
Under chronic cyclic hypoxia, hypoxic PANC-1 cells have a comparable doubling time with their normoxic counterparts, whereas hypoxic MCF7 cells show a massive increase in doubling time when compared to their normoxic counterparts. Both hypoxic cell lines developed EMT-like phenotypes as well as doxorubicin resistance. According to the findings of miRNet, 6 and 10 miRNAs have been shown to play an important role in enriching six hallmarks of pancreatic cancer in the tenth and twentieth cycles of hypoxia, respectively, while 7 and 11 miRNAs have been shown to play an important role in enriching the four hallmarks of breast cancer in the tenth and twentieth cycles of hypoxia, respectively.

Conclusions
miR-221, miR-21, miR-155, and miR-34 were found to be involved in the potentiation of hypoxic PANC-1 hallmarks at both the 10th and 20th cycles, while miR-93, miR-20a, miR-15, and miR-17 were found to be involved in the potentiation of hypoxic MCF7 hallmarks at both the 10th and 20th cycles. This variation in miRNA expression was also connected to the emergence of an EMT-like phenotype, alterations in proliferation rates, and Doxorubicin resistance. Indeed, further research is needed since the particular mechanisms that govern these processes are unknown.

POSTER | N°26

Identification of a New Hypoxic Biomarker in the MCF7 Breast Cancer Cell Line

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Abstract

Hypoxia mediates cancer hallmarks and results from reduced oxygen level due to irregularities in tumor vascularization or when tumor size prevents oxygen diffusion and triggers angiogenesis to compensate for low oxygen. The main aim of this study is to discover new key player biomarkers in tumor hypoxia especially in long term exposure to hypoxic conditions by identifying gene expression changes that occur in breast cancer cells under hypoxic conditions. Breast cancer cells (MCF7) were exposed to 8-hour hypoxic episodes (<1% oxygen) three times a week for a total of 60 episodes (track 2) and once a week for 72 hours (track 3) for a total of 12 episodes over a three-month period. Gene expression changes were profiled using hypoxia RT- PCR array. To evaluate the effect of Hypoxia on angiogenesis, a migration assay was conducted after 60 episodes of hypoxia in track 2 and 12 episodes of hypoxia in track 3. Genes that were up-regulated in track 2 and 3 included HNF4A, SLC2A3, SLC16A3, EGLN1 and IGFBP3 genes. Interestingly, the HIF-1 signalling pathway was the pathway that was up-regulated the most in both hypoxia modalities. A notable gene (Hepatocyte Nuclear Factor 4) was up-regulated by 14.95 folds after 60 hypoxic shots and 32.71 folds after 12 hypoxic shots. The ability of cells to migrate was increased after exposing cells to both tracks although track 3 were the most affected. This study identified HNF4A as a potential biomarker in tumor hypoxia and a key player in the response of the MCF7 breast cancer cells to long term hypoxia.

POSTER | N°27

Synthesis and antiproliferative activity evaluation of 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives of Nortopsentin

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Introduction

Among marine bis-indole alkaloids, Nortopsentins represent promising lead compounds due to their in vitro cytotoxicity. Our research group synthesized new analogues, such as thiazolyl derivatives, which showed GI50 values in the micromolar to sub-micromolar range against the NCI full panel of 60 cancer cell lines. Interestingly they also caused CDK1 inhibition with IC50 values comparable to Roscovitine and Purvalanol A, suggesting further studies in malignancies with aberration of this kinase, such as pancreatic ductal Adenocarcinoma (PDAC).

Materials and methods

New Nortopsentin analogues (1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives) were synthesized and evaluated activity against a panel of PDAC cell lines. In vitro antiproliferative activity and anti-migratory activity were studied by sulforhodamine-B and wound-healing assays, using seven PDAC cell lines (BxPC-3, Suit-2, Capan-1, PaTu-T, Panc-1, and gemcitabine-resistant Panc-1 (Panc-1-GR)) and the primary culture PDAC-3. Analysis on cell cycle and apoptosis were performed by cytofluorimetry.

Results

Five compounds reported GI50 values in the micromolar range. The most active oxadiazole derivative **(1b)** showed GI50 values in the range 1.4-5.0 μM, while the most active thiadiazole derivative **(3b)** showed GI50 values in the range 1.9-7.6 μM. We also found a remarkable reduction of migration in all the cancer cell lines tested. In particular, after 24 hours, the migration percentage of cells treated with 4-fold GI50 concentration values of **1b**, and **3b** were respectively 23% and 34% in PDAC-3; 49% and 42% in Panc-1-GR; 67% and 61% in Panc-1; 48% and 45% in Capan-1. Cell cycle analysis showed an increase in G2/M cells and a decrease in the G1 phase. In PDAC-3 cells, the compound **1b**, tested at concentrations of 2 μM and 4 fold GI50, decreased the G0/G1 cells respectively from 59% to 51% and 27%, increased the G2/M cells from 16% to 24% and 49%, while decreased the cells in S phase from 24% to 23% and 22%.

Conclusion

Compounds **1b** and **3b** showed promising antitumor activity against the seven cancer PDAC preclinical models, supporting further studies to elucidate their mechanism of action, such as the inhibition of CDK1 activity, and for the development of similar compounds as new treatment against PDAC.

POSTER | N°28

Synthesis and pharmacological evaluation of a 1,2,4-triazine-based library as selective pyruvate dehydrogenase kinase 1 (PDK1) inhibitors

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Introduction

Among the different metabolic altered types of cancer, pancreatic ductal adenocarcinoma (PDAC) has emerged as one of the most aggressive and resistant malignancies.[1] Despite efforts made to develop more effective therapeutic strategies for PDAC, its incidence constantly rises. Therefore, there is an urgent need for the development of more effective treatments and agents that redirect energy metabolism from respiration to glycolysis by targeting key regulators of cancer cell metabolism. Particularly, the pyruvate dehydrogenase kinases (PDKs), involved in the Warburg effect, are linked to tumor aggressiveness, proliferation, and chemotherapy resistance. [2]

Materials and methods

Herein we reported a new series of 1,2,4 triazine compounds, characterized by in vitro antitumor activity in 2D and 3D cell models of PDAC, assessed by MTT assay. In addition, a cell-free chemoluminescent assay was carried out to evaluate the inhibition of the catalytic activity of PDKs, further confirmed by molecular docking. Moreover, preliminary in vivo studies were conducted in a murine Lewis Lung Carcinoma (LLC) tumor model.

Results

The synthesized compounds were able to hamper the enzymatic activity of PDKs, properly placed inside the nucleotide-binding pocket of the kinases. As a proof of concept of the involvement of the compounds in the altered metabolic pathway, detailed cellular mechanistic studies confirmed the derivatives' ability to stabilize the PDC/PDK axis, thus determining mitochondrial damage, increasing cellular oxygen consumption, decreasing lactate production, and leading to cancer cell death by reversing the Warburg effect. These promising biochemical results were also confirmed by the in vivo studies, which demonstrated a higher percentage of reduction of tumor mass, similarly to cisplatin and gemcitabine, but with a lower body weight loss.

Conclusion

A new library of triazine-based derivatives has been successfully synthesized and evaluated on 2D cultures and 3D spheroids of PDAC RAS wild-type and mutant cells. The in silico, in vitro, and in vivo results showed the ability of the tested derivatives to reduce cancer growth and counteract the cancer metabolic imbalance through the inhibition of PDK. Collectively, the data highlights the promising anticancer potential of these novel derivatives, clinical candidates for combatting KRAS-mutant pancreatic ductal adenocarcinoma.

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POSTER | N°29

Immune response and liposomal nanoparticles: it's a match

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Due to their physical and chemical characteristics, nanoparticles such as liposomes, are expected to interact with the tumor immune system. For instance, immunosuppressive cells such as myeloid-derived suppressor cells (MDSC), regulatory T cells (Treg) or lack of infiltrated T lymphocytes have all been described to play a major role in the therapeutic failure of immune checkpoint inhibitors. The aim of this proof-of-concept study was to compare in mice the impact on immunity of blank stealth immunoliposomes grafted with trastuzumab (ANC) and blank stealth liposomes (Lipo).

Balbc mice were treated (IV route) by either saline (control), Lipo or ANC QW for 5 consecutive weeks. Blood samples were withdrawn QW for immune-monitoring and spleen resection was performed on Week 6.

Antibodies were used for flow cytometry analysis for immune cells characterization (i.e., B cells, T cells (CD4 & CD8), Treg, MDSC). Multiparameter analysis was performed on a Gallios flow cytometer and finally analyzed using the Kaluza® software. Statistical analyses were performed on MedCalc 17.2.1. Software.

No statistically significant difference was found in MDSCs between treated mice vs. control, both in spleen and in blood ($p > 0.1$). Regarding spleen T cells, there was a statistically significant difference with both ANC and Lipo vs. control for each T cells cluster. Regarding blood T cells, a statistically significant difference with both ANC and Lipo was found vs. control from Week 2 for each T cells cluster as well. Of note, no difference was evidenced on the immune cell repertoire between ANC and Lipo.

Overall, this study highlights that immune response triggered by liposomal nanoparticles is significant and sustained throughout time. The next step of this pilot study is to replicate those findings in tumor-bearing animals and check the impact of liposomes on the immune cells in the tumor micro-environment. Should this be confirmed, then it will pave the way for combining liposomal nanoparticles with immune checkpoint inhibitors to reshape the tumor immune landscape.

Keywords:
nanoparticles, liposomes, immunotherapy, mice model, immunomonitoring

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POSTER | N°30

Diagnostic and evolutionary profiles of adrenocortical tumor, about 30 cases

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Abstract

Adrenocortical tumor is a rare malignant tumor of adrenal location. With a poor prognosis that can be improved by rapid diagnosis and adequate early management, hence the importance to evoke this etiology in front of any atypical adrenal mass or any suggestive clinical or biological context.

The objective of our modest work is to report through a retrospective study the profile of adrenocortical tumors treated in our service over a period of 13 years from 2007 to 2020.

Thirty (30) patients were collected with a middle age of 45 years (27 to 74 years) with a slight female predominance of 60%. A family history of neoplasia was noted in 5 patients (16.66%) and breast neoplasia in a single patient. The most frequent mode of revelation is represented by abdominal pain observed in 56.66% of cases. Hypercortisolemia is found in 40% of cases. Morphologically, the size of the tumor varies from 66 to 180 cm with an average of 110 cm. At least one metastasis was found in 10 patients (33.33%), dominated by hepatic location. 50% of our patients benefited from surgical excision of the tumor and 6.66% underwent a biopsy only. 77% of our patients received adjuvant treatment with mitotane supplemented by chemotherapy in 30% of cases. Only two patients received adjuvant radiotherapy (6.66%). The evolution was marked by remission in 33.33% of cases with an average survival of 5 years and death noted in 50% of patients.

In conclusion : Adrenal carcinoma can be seen at any age with a peak frequency in the fifties, affects both men and women with a slight female predominance. Large tumors whose treatment is surgical associated with neoadjuvant treatment in the majority of cases. Unfavorable prognosis, but optimized management can improve survival.

POSTER | N°31

Isolation and characterization of H23 clones resistant to the KRASG12C inhibitors sotorasib and adagrasib

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Introduction

KRAS mutations represent the most common gain-of-function reported in many tumors and they account for 25%-30% of NSCLC adenocarcinomas. Despite their well-known role in carcinogenesis, a long history of unsuccessful treatment options is documented. Recently, two drugs, sotorasib, and adagrasib have been developed to target the KRASG12C point mutations. Regardless of their initial success, as reported for other specific targeted agents, the resistance phenomenon has been reported in both in vitro cell models and patients.

Materials and methods

We employed H23 NSCLC cells carrying KRASG12C point mutation to study acquired resistance mechanisms to the drugs. Resistant clones were obtained by high-dose drug approach. Genetic analyses were performed by NGS assay (TruSight Oncology 500 panel, Illumina). Cell proliferation, clonogenic assay, and cell migration have been assessed.

Results

H23 NSCLC cells are sensitive to both drugs with IC50 in the range of low nanomolar concentration. After 5-6 months of chronic treatment, resistant clones able to grow at 1 and 0.5 μM concentrations for sotorasib and adagrasib respectively were isolated. These clones are characterized by cross-resistance, and high migratory properties compared to H23 parental cells. Genetic analysis revealed that KRASG12C mutation was maintained and no secondary KRAS mutations were detected. The high level of ERK1/2 phosphorylation observed in clones suggested KRAS-independent resistance mechanisms.

Different genetic alterations were found by NGS analysis: mutations in the β-catenin-axin1 signaling and in the untranslated region of the ETV5 transcription factor, and amplifications of NRAS, FGF6, and FGF23 genes. The significance of these alterations is under evaluation.

Conclusion

KRASG12C point mutation is an attractive target in NSCLC, but drug resistance is irreversibly emerging, limiting the duration of the therapy.

Resistant clones are characterized by a high migration index and ERK1/2 phosphorylation and the lack of secondary KRAS mutations suggested a resistance mechanism KRAS-independent. No alteration of the MAPK pathway has been reported, and different mutations have been detected in clones, evidencing that resistance could be a consequence of different mechanisms. The β-catenin-axin1 and FGF 6-23 gene alterations could suggest the rationale for the combination of KRASG12C inhibitors with agents directed to these signaling.

POSTER | N°32

Inflammatory risk factors of the ENT sphere in nasopharyngeal cancer

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Introduction

Nasopharyngeal cancer is a cancer of multifactorial etiology, involving several environmental and genetic factors. A possible link of this cancer with inflammation of the ENT sphere has been described in the literature.
Indeed, a history of inflammatory pathology such as rhinitis, pharyngitis and angina seem to be incriminated in the occurrence of this cancer.

The objective of our work is to study the relationship between the history of inflammatory pathologies of the ENT sphere and the occurrence of nasopharyngeal cancer in population of westen Algeria.

Patients and Methods

This is a case-control study carried out on all incident cases of nasopharyngeal carcinoma, followed at two hospital structures in Oran, Algeria during the period 2016-2018. The data was collected from a pre-established standardized questionnaire, by direct interview with patients. Data entry and statistical analysis were performed using Epidata software.

Results

The study included a sample of 120 patients and 120 controls matched on age and sex. The average age of patients is 45.2 ± 2.5 years with a male predominance. Two-thirds of patients live in an urban area (70.8%). The univariate statistical analysis of the data shows that chronic inflammatory pathologies of the ENT sphere (Angina, Otitis, Rhinitis) are not statistically associated with the risk of occurrence of NPC ($p>0.05$).

Conclusion

Chronic inflammatory pathologies are not risk factors in the occurrence of nasopharyngeal cancer in our study population. A better knowledge of the other risk factors would make it possible to put in place preventive measures.

Keywords:
Nasopharyngeal cancer, inflammatory pathologies, ENT

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POSTER | N°33

Next-generation sequencing mutational analysis in pancreatic cysts fluid

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Introduction

The management of pancreatic cystic lesions is a medical challenge as pathological features are associated with different clinical issues difficult to identify before surgery. Molecular characteristics of neoplastic tissues allow to distinguish cysts regarding their malignant potential for which cysts fluid analysis has demonstrated its relevance. We set up a national multicentric study to define the genetic profile of cystic fluids according to their clinicopathologic characteristics.

Material & Methods

The analysis of a 50-genes panel using next generation sequencing was performed on DNA extracted from cystic fluid of 120 patients collected from February 2020 to June 2021. All patients harbored cysts over 15 mm.

Results

A total of 93 DNA samples were suitable for NGS analysis. Mutations were detected in 71 of 93 DNAs, mainly on KRAS, GNAS, TP53, RAF, CTNNB1 and POLD1 genes. BRCA2 and ATM were also involved in several cases. Unique variants were finally detected. These mutations were highly associated with the presence of dysplastic or cancerous cells in the corresponding cysts, that were surgically removed.

Discussion

This study takes place after a pilot study that showed a good concordance of genomic profile between cystic fluid and cyst wall biopsy (1). The results reinforce the interest of including the DNA profiling of cystic fluid before entering patients in a surgical program in addition to new imaging approaches. It allowed to distinguish benign from malignant and pre-malignant lesions, thus encouraging to propose a systematic genomic evaluation of cysts fluid before surgical decision.

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Laquière AE, Lagarde A, Napoléon B, … P, Olschwang S. Genomic profile concordance between pancreatic cyst fluid and neoplastic tissue. World J Gastroenterol. 2019;25:5530-5542

POSTER | N°34

Early onset biliary tract cancers (EOBTCs): outcomes, clinical and molecular data of a single-centre case series

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Introduction

Biliary tract cancers (BTCs) are commonly age-related although recent evidence suggests an increase in the incidence rate in younger people.

Material and methods

We retrospectively collected epidemiological, pathological and molecular data of EOBTCs, defined as under 50 years old at diagnosis, followed at the Pisa University Hospital between 2011 and 2021.

Results

Forty-two patients (pts) were identified. Median age at diagnosis was 46 years old (range 26-50). At a median follow up of 66.6 months (mo) (95% confidence interval [CI] = 47.2 to 112.9 mo), 9 pts were still alive. Median overall survival was 18.5 (95% CI 10.1 to 29.4) mo in the entire cohort and 9.0 (95% CI 6.5 to 16.4) mo for pts with stage IV disease at diagnosis. Fifteen (35.7%) pts had a family history of cancer and 15 (35.7%) had a personal history of risk factors related BTCs' development. Actionable alterations were identified in 20% (4/20) of pts (3 with intrahepatic cholangiocarcinoma and 1 with extrahepatic disease) in which we performed a FoundationOne® test: 2 pts showed a FGFR2-WAC fusion, 1 had a IDH1 R132L mutation, and 1 presented a BRAF non-V600E mutation. No HER2, BRCA and PALB2 alterations were detected. Among the 19-pts tested, 18 had a mismatch repair (MMR) proficiency and 1 had MMR deficiency; he refused a genetic assessment. Three pts benefited from a target treatment informed by their molecular profile in lines equal or subsequent than the 2nd (2 pts received FGFR inhibitors and 1 Ivosidenib); mOS from diagnosis of metastatic disease was 9.6 mo in pts with no molecular target and 21.3 mo in those with one (HR= 0,4422 95% CI 0,18-1,07 p=0.13) receiving tailored targeted agents.

Conclusions

In our case series, actionable somatic alterations can be identified in EOBTCs and are linked to potentially relevant survival benefit when targeted treatment is available. Since EOBTCs represent an emerging issue, an extensive molecular profiling as well as availability of genetic counselling is recommended. In addition, dedicated studies on molecular biology and therapeutical management of EOBTCs are warranted.

POSTER | N°35

An immune-related gene expression profile predicts the efficacy of adding atezolizumab to first-line FOLFOXIRI/bevacizumab in metastatic colorectal cancer: A translational analysis of the phase II randomized AtezoTRIBE study

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Background

AtezoTRIBE study demonstrated that adding atezolizumab to first-line FOLFOXIRI/bevacizumab prolongs progression-free survival (PFS) of metastatic colorectal cancer (mCRC) patients, with a modest benefit among proficient mismatch repair (pMMR) tumours. DetermaIO is an immune-related 27-gene expression signature able to predict benefit from immune checkpoint inhibition in triple negative breast cancer (TNBC). We investigated the predictive impact of DetermaIO in mCRC patients enrolled in AtezoTRIBE.

Methods

AtezoTRIBE phase II trial randomized 1:2 mCRC patients unselected for MMR status to receive FOLFOXIRI/bevacizumab (control arm) or FOLFOXIRI/bevacizumab/atezolizumab (atezolizumab arm). Gene expression was measured using RT-qPCR by DetermaIO™ on RNA purified from FFPE blocks of pre-treatment tumour samples (132/218 enrolled patients [61%]). IO score was calculated using the proprietary algorithm. Tumours were dichotomized as IO+ or IO- according to a pre-established cut-point (0.09), previously set in independent TNBC datasets, and as IOopt+ or IOopt- according to an exploratory optimized cut-point (IOopt).

Results

DetermaIO was successfully determined in 122 (92%) cases, with 23 (27%) IO+. Patients with IO+ tumours achieved higher PFS benefit from the atezolizumab arm than those with IO- tumours (HR:0.39 versus 0.83, P for interaction=0.066). In pMMR tumours (N=110), a similar trend was observed (HR:0.47 versus 0.93, P for interaction=0.139). In the overall population (N=122), according to the computed IOopt cut-point (0.277), 16 (13%) tumours were IOopt+. IOopt+ derived higher PFS benefit from the atezolizumab arm than IOopt- ones (HR:0.10 versus 0.85, P for interaction=0.004). Similar results were observed in the pMMR subgroup.

Conclusions

DetermaIO may be useful to predict benefit from the addition of atezolizumab to first-line FOLFOXIRI/bevacizumab in mCRC patients. The exploratory IOopt cut-point should be validated in independent mCRC cohorts.

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POSTER | N°36

Prospective evaluation of emergent RAS and BRAF mutations in pre-treated metastatic colorectal cancer patients candidate to anti-EGFR re-treatment: preliminary findings from the PARERE study

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Background

Retreatment (re-tx) with anti-EGFRs is a promising strategy in RAS/BRAF wild-type (wt) anti-EGFR pre-treated metastatic colorectal cancer patients (mCRC pts), provided that no mechanisms of acquired resistance to anti-EGFRs are found in circulating tumor DNA (ctDNA). The CHRONOS trial showed a 31% prevalence of RAS/BRAF mutations (mut) in ctDNA of pts candidate to anti-EGFR re-tx. We are now conducting a phase II randomized study, PARERE, to compare panitumumab followed by regorafenib versus the reverse sequence in anti-EGFR pre-treated chemorefractory pts with RAS/BRAF wt ctDNA. Here we show initial results from the molecular screening.

Methods

RAS/BRAF wt mCRC pts who achieved ≥6 months (mo) benefit with a previous anti-EGFR based tx and received ≥1 subsequent anti-EGFR free tx lasting ≥4 mo were eligible. ctDNA was analysed with the Next Generation Sequencing Ion TorrentTM OncomineTM cfDNA Colon assay, enabling parallel profiling of 14 genes.

Results

101 pts were screened and ctDNA was successfully assessed in 90 (90%). 25 (28%) harboured KRAS/NRAS/BRAF V600E mut. Within the RAS/BRAF wt cohort (n=65), mut in at least another gene were found in 49 pts (75%). TP53, APC, PIK3CA, FBXW7, GNAS, EGFR, AKT and SMAD4 mut occurred in 39 (60%), 24 (36%), 8 (12%), 7 (11%), 2 (3%), 1 (1%), 1 (1%) and 1 (1%) cases, respectively. No mut in ERBB2, CTNNB1 and MAP2K genes were found. The overall limit of detection was 0.07% [95% CI: 0.06-0.09%] and the median variant allele fraction of RAS/BRAF genes was 0.32% [95% CI: 0.23-1.70%]. The median anti-EGFR free interval did not differ between the group with RAS/BRAF wt and RAS/BRAF mut ctDNA (13.8 [95% CI: 12-18.3] and 16.9 mo [95% CI: 10.6-24.6], respectively, p=0.70).

Conclusions

This is the largest series of pts prospectively screened for anti-EGFR re-tx. About one third of pts bear RAS or BRAF mut in their ctDNA. Anti-EGFR free interval is not a valuable surrogate of ctDNA mut status, thus supporting liquid biopsy as a selection tool for clinical trials in this setting and for the use of anti-EGFR re-tx in the real life.

Keywords:
Metastatic colorectal cancer, anti-EGFR re-treatmentliquid, next generation sequencing, biopsy, circulating tumor DNA,

References:
Siravegna et al., Nature Medicine 2015, Cremolini et al., Jama Oncology 2019, Moretto et al, Clinical Colorectal Cancer 2021, Sartore-Bianchi et al., Nature Medicine 2022

POSTER | N°37

Prognostic value of new molecular-integrated classification in early-stage endometrial cancers treated with adjuvant vaginal brachytherapy: a retrospective experience

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Introduction

Vaginal brachytherapy is currently recommended as adjuvant treatment in patients with high-intermediate risk endometrial cancers. It is hypothesized that the use of the new molecular-integrated classification to determine the adjuvant treatment would overcome traditional clinical risk group assessment reducing overtreatment and some undertreatment. A separated group of women with high-risk factors like substantial lymph-vascular space invasion (≥5 LVSI-involved vessels) or p53 overexpression could possibly benefit from a more aggressive treatment to maximize local and distant control

Materials and methods

We retrospectively analysed 79 patients with endometrial cancer who underwent extrafascial hysterectomy with or without lymphadenectomy and adjuvant high-dose-rate (HDR) vaginal brachytherapy (VBT) in our Institution (Azienda Ospedaliera Universitaria Pisana, Division of Radiation Oncology). All women received VBT to the vaginal vault with a vaginal cylinder and the VBT dose was 21 Gy in 3 fractions of 7 Gy. Based on histopathology reports, patients were assigned to the molecular-integrated unfavourable risk-profile group in case of substantial lymph-vascular space invasion (≥5 LVSI-involved vessels) or p53 overexpression.

Results

In our analyses, any disease recurrence (both local or distant) was observed in the unfavourable molecular-integrated risk group: there was a statistically significant correlation between molecular-integrated unfavourable risk-profile and pelvic recurrence (p=0.002), distant recurrence (p= 0.017), vaginal recurrence (p=0.02) and nodal pelvic recurrence (p=0.006). Patients with p53 overexpression had greater risk to develop local recurrent disease (p=0.041) or distance disease (p=0.065); we also found a statistically significant relationship between non-endometrioid tumours and frequency of case overexpressing p53 (p<0.001). Substantial LVSI was a very strong prognostic factor for pelvic regional recurrence (p= 0.001) and distant metastasis (p<0.001)

As would be expected, non-endometrioid tumours were associated with poor outcomes (p<0.001) and the presence of Estrogen and Progesterone receptor expression was correlated with a better prognosis.

Conclusion

Different molecular-integrated risk groups show significant differences in term of local and distant recurrence rate: our experience suggests that the new molecular-integrated classification could play an important role in determination of a more tailored adjuvant treatment in patients affected by early-stage endometrial cancers

Keywords:
Endometrial cancer, p53, lymph-vascular space invasion, brachytherapy,molecular-integrated classification

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