

SARCOMA

HMGA1/E2F1 AXIS AND NFKB PATHWAYS REGULATE LIPOSARCOMA PROGRESSION AND TRABECTEDIN RESISTANCE.

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ABSTRACT

Conventional lipoma is characterized by extensive HMGA1 aberrations, suggesting a role of the protein in the mechanisms of liposarcoma progression. We found that the overexpression of HMGA1 protein is strongly involved in the mechanism of cell proliferation, motility and invasion of the high-grade liposarcoma. These results were confirmed in vivo in liposarcoma specimens confirming the role of HMGA1 protein in liposarcoma progression. The treatment with trabectedin down-regulates HMGA1 and E2F1, as well as E2F1-downstream targets vimentin and ZEB1, in the sensitive myxoid liposarcoma cells but not in resistant counterpart cells suggesting a critical role of HMGA1/E2F1 axis in the regulation of the mesenchyme compartment in liposarcoma. These data obtained in in vitro growing liposarcoma cell lines were confirmed in vivo in patients' tumor biopsies taken from two patients who received trabectedin treatment as neoadjuvant chemotherapy in ISG-ST5-10-01 trial (NCT01710176, EUDRACT 2010-023484-17). Interestingly, in these specimens we found a translocation of vimentin from the cytoplasm to the nuclei following trabectedin therapy. Furthermore, trabectedin treatment induces apoptosis in sensitive cells inhibiting NFkB pathway but not in resistant cells. The inhibition of NFkB causes the accumulation of p65 protein at the cytoplasm and down regulates HRGb1 expression that, in turn, inhibits p-HER3, and re-sensitizes the cells to trabectedin treatment. These data provide the rationale for combining NFkB inhibitors with trabectedin in liposarcoma patients who have become resistant to the drug.

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A QUINOLINE-BASED DNA METHYLTRANSFERASE INHIBITOR AS A POSSIBLE ADJUVANT IN THE THERAPY OF BONE SARCOMAS.

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The need of new therapeutic strategies against bone sarcomas, continues to represent a primary goal for patients refractory to conventional chemotherapy. Osteosarcoma (OS) and Ewing's sarcoma (ES) represent the most common primary bone tumors and share a mesenchymal lineage origin where a transformation occurs during the stage of stem cells (MSCs) and/or progenitors. The loss of differentiation is a widespread biological feature of these tumors and results in worsening of prognosis. A growing body of evidence shows how defects at the level of epigenetics and genetics interplay is fundamental for sarcomagenesis. Thus, restoring differentiation through epigenetic reprogramming might be exploited for therapy improvement. This study demonstrates that the novel non-nucleoside DNMT inhibitor (DNMTi) MC3343 affects tumor proliferation by blocking tumor cells in G1 or G2/M phases and induces differentiation. In ES, the treatment elicits differentiation through induction of specific terminal neural markers. In OS cells, MC3343 increased both matrix mineralization and expression of genes specifically related to osteoblastogenesis. Even though MC3343 shares its anti-proliferative effect with 5azadC, the conventional DNA demethylating agent, the effects on cell differentiation are distinctive for the MC3343 compound. Induction of mature osteoblast phenotype coupled with sustained cytostatic response was further confirmed in vivo when MC3343 was administered to patient-derived OS xenograft (PDX-OS) mice. In addition, MC3343 displays synergistic effects with the major chemotherapeutic agents that interact with DNA and are commonly used in the treatment of OS. In particular, MC3343 increases DXR-stable bounds to DNA, with a consequent sustained DNA damage and increased cell death as a result. Overall, this non-nucleoside DNMTi acts as a specific and effective novel agent that might be considered as a therapeutic tool in patients with poor response to pre-adjuvant chemotherapy.

SARCOMA

GENERATION OF NEW INHIBITORS OF TUMOR MICROVESSEL DENSITY AND VASCULAR INFILTRATION BY SARCOMA CELLS.

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Despite significant progress regarding potential therapeutic targets aimed at improving patient survival, patients affected by sarcoma frequently die for systemic spread of the disease, mainly to the lungs. Thus, there is a pressing need to develop new targeted approaches. Human Formyl-Peptide Receptor type-1 (FPR1), originally identified in myeloid cells, elicits many responses upon ligation to formyl-peptide ligands derived from bacteria or mitochondria of eukaryotic cells, including locomotion and release of cytokines and proteolytic enzymes. Recently, FPR1 has been shown to be expressed in several non-myeloid cells, and accumulating evidence demonstrates that FPR1 is involved in progression of solid tumors. Previously, we described linear peptide antagonists of FPR1 that inhibit cell migration. To develop enzyme-resistant analogues, we applied the Retro-Inverso (RI) approach, whereby the topology of the side chains is maintained by inverting the sequence of the peptide and the chirality of all residues. Among these, the peptide Ac-(D)-Tyr-(D)-Arg-Aib-(D)-Arg-NH₂ (RI-3) which is stable in human serum, is a not cytotoxic, nanomolar competitor of N-formyl-Met-Leu-Phe for binding to FPR1. RI-3 inhibits migration. By organotypic 3D co-cultures of sarcoma spheroids dropped into collagen matrices embedded with fibroblasts, we have found that RI-3 prevents spheroid size increases over time in matrices. Furthermore, RI-3 inhibits at 10 nM concentration, trans-endothelial migration of primary sarcoma cells. In vivo, when i.p.-administrated at 6 mg/Kg, RI-3 reduces microvessel density in tumors formed by primary human sarcoma cells injected subcutaneously in the flanks of nude mice, decreases the number of circulating tumor cells in the murine blood samples and prevents pulmonary metastasis. Thus, RI-3 represents a promising lead for anti-metastatic drugs as it prevents three key events occurring during the metastatic process: the matrix invasion, the formation of a capillary network and the entry into bloodstream.

SARCOMA

THREE-DIMENSIONAL ORGANOTYPIC CO-CULTURES OF SARCOMA SPHEROIDS WITH CELLULAR COMPONENTS OF THE TUMOR MICROENVIRONMENT: A MODEL TO TEST THE EFFICACY OF NEW ANTI-TUMOR DRUGS.

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BACKGROUND:

Preclinical models that represent clinical behaviour in sarcoma are essential to improve patient outcomes. Today, most of available models neither represent sarcoma heterogeneity nor feature the interactions between tumor cells and tumor microenvironment (TME). TME cells play a pivotal role in inducing immune tolerance and promote tumor growth, matrix invasion, and metastatic dissemination. Noteworthy, TME cells are genetically more stable than tumor cells, representing a good target for development of new therapeutic strategies with reduced risk of resistance. In this context, 3D-organotypic co-cultures may be considered most biologically relevant in vitro models to recapitulate key aspects of architecture of solid tumors in an in vivo-like environment.

PROPOSAL:

Our focus is to expand the knowledge of sarcoma in the context of TME, using 3D-organotypic co-cultures of sarcoma spheroids dropped into collagen matrices embedded with fibroblasts and TME cells and, later on, in vivo models that can be maintained and sampled in real time. Results will allow to identify the contribution of TME cells in sustaining proliferation, invasion, and trans-endothelial migration of sarcoma cells. Once we have identified the TME cellular activities contributing to sarcoma progression, we will attempt a re-education of TME cells with immune checkpoint blockade therapeutics, or drugs recently developed by applicants, capable to prevent monocyte recruitment and macrophage M2-polarization, in combination with conventional chemotherapeutics.

PRELIMINARY RESULTS:

By 3D-organotypic co-cultures, we found that spheroid size increases over time in matrices. Sarcoma cells and fibroblasts spread in the matrix, moving towards each other already after 24h co-culture, continuing to spread up to 7 days. The presence of M2-like-macrophages enhanced cell trafficking, increasing both spheroid size and spreading of sarcoma cells. Furthermore, peptide inhibitors of macrophage M2-polarization caused an appreciable decrease of spheroid size and spreading of sarcoma cells into matrices. This proposal is supported by the Italian Sarcoma Group.

SARCOMA

NEUTROPHILS EXERT AN ANTI-TUMORAL ROLE IN 3MCA-INDUCED SARCOMA CARCINOGENESIS.

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PURPOSE:

The view of neutrophil as a cell involved only in the early phases of inflammation has been challenged in the last years, so that neutrophils are now considered key players in the orchestration of the immune response. To determine neutrophil contribution to tumor development, previous studies often relied on the poorly effective antibody-based neutrophil depletion in transplantable tumor models, but rigorous in vivo evidence assessing the neutrophil role in cancerogenesis is missing.

METHODS:

We investigated this issue using a model of chemically-induced cancer (3-MCA induced sarcoma) and taking advantage of a genetic model of neutrophil deficiency (*csf3r*^{-/-} mice).

RESULTS:

Neutrophil deficiency was associated with increased susceptibility to sarcoma, and tumor microenvironment from *csf3r*^{-/-} mice displayed protumoral features (e.g. increased frequency of M2 macrophages, reduced IFN γ concentration and skewed T cell polarization). In addition, neutrophil density within tumor significantly correlated with reduced proliferation rate of tumor cells in immunocompetent mice. Importantly, adoptive transfer of naïve neutrophils reduced tumor growth in *csf3r*^{-/-} mice, restoring normal IFN γ levels. Additionally, IFN γ depletion completely abolishes sarcoma protection observed in *csf3r*^{+/+} mice.

DISCUSSION:

Collectively, our data indicate that genetic deficiency of neutrophils affects the anti-tumor response and is associated with increased susceptibility to chemically-induced cancerogenesis.

CONCLUSIONS:

Until recently neutrophil function was mostly related to acute inflammation and defense against pathogens. We (and others) have challenged this dogma and demonstrated that neutrophils represent an essential component in the control of tumor onset and development.

SARCOMA

ESTABLISHMENT OF A PATIENT-DERIVED XENOGRRAFT OF SOFT-TISSUE SARCOMA IN A ZEBRAFISH MODEL: MOLECULAR AND PHARMACOLOGICAL PROFILE.

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BACKGROUND:

Soft Tissue Sarcoma (STS) and Bone Sarcoma (BS) are a heterogeneous group of mesenchymal tumors accounting almost 1% of all adult solid tumors. Pre/clinical data focusing on this poorly explored disease are still lacking. Thus there is the need to identify novel molecular markers for diagnostic purposes and to open doors to new therapeutic horizons. In this regard, patient-derived specimens represent precious tools to study the tumor pathophysiology and pharmacology. Furthermore, Zebrafish recently emerged as a promising xenograft tumor model could represent a potentially powerful system for preclinical studies of STS primary cultures.

OBJECTIVES:

The project purpose is the characterization of gene expression and pharmacological profiles of selected STS histotypes. The analysis will be carried out on patient samples, immortalized cell lines and primary cultures both in vitro with the use of 2D culture and 3D collagen-based scaffolds and in vivo with zebrafish. The results will be correlated with clinical outcomes. Research methodology: sarcoma tissues will be collected to perform NGS analyses. Results will be related to clinical-pathological status in order to identify a panel of possible prognostic markers and markers of response according to patients treatment. Moreover, STS cell lines and primary cultures will be treated with chemotherapeutic, biologic and immunotherapeutic agents both in vitro and in vivo with xenograft generation into Zebrafish and the different behavior will be related to NGS profile.

EXPECTED RESULTS:

The characterization of gene expression patterns and the correlation with clinical outcomes might help to find markers able to predict the prognosis and response to therapy. Furthermore, the pharmacological profile will give evidence on tumor sensitivity to different treatments and could underline possible mechanism of chemoresistance.

CONCLUSIONS:

The project is pioneering and its results can be a starting point for further research on classifying sarcomas as well tailoring patient treatment. Proposal supported by ItalianSarcomaGroup.

SARCOMA

ZYXIN ROLE IN EWING SARCOMA.

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Ewing sarcoma (EWS) is a rare and aggressive tumor of the bones, affecting children and adolescents. EWS cells typically express a disease-specific oncogenic chimeric transcription factor, usually EWS-FLI1. In EWS cells is also highly expressed the glycoprotein of membrane CD99, whose presence is necessary for the maintenance of tumor's malignancy. In this context recently has attracted attention the role of the LIM protein zyxin, which is normally localized in cytoskeleton structures but can also shuttle in the nucleus and regulates gene expression. In EWS cells zyxin assumes a tumor suppressor role and it is silenced by EWS-FLI1. In the present study, through the analysis of a coorte of EWS patients, we confirm the low expression of the protein in this tumor and find out a positive prognostic role of zyxin. According to these results, in EWS cell lines zyxin is slightly expressed and regulates negatively the cellular migration and proliferation. Then we investigate zyxin potential interactions with CD99 and EWS-FLI1. Precedents studies have seen zyxin as mediator of Ewing sarcoma cell death induced by engagement of CD99 with a specific antibody (MAb O662). Here we observe that the treatment with O662 MAb or silencing of CD99 leads to an increase in zyxin levels, its nuclear translocation and a major rearrangement of actin cytoskeleton. In addition, we prove that upon O662 MAb treatment, zyxin physically interacts with CD99 and shuttles to the nucleus where it can prevents the binding of EWS-FLI1 to the promoters of its gene target. In conclusion, EWS-FLI1 and CD99 repress zyxin's activity, but upon inhibition of CD99 zyxin shifts in the nucleus and regulates negatively EWS-FLI1 functions. Grants: AIRC_IG18451 to KS.

SARCOMA

PREDICTIVE VALUE OF EXOSOMIC MIRNAS DURING TREATMENT WITH CONVENTIONAL AND TARGETED THERAPIES IN SARCOMAS -A LIQUID BIOPSY APPROACH.

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BACKGROUND:

The lack of predictive biomarkers of response jeopardizes the introduction of precision medicine in sarcoma treatment. Genetic lesions and cellular mechanisms responsible for primary and acquired resistance are often difficult to identify and to correlate with relevant clinical outcomes. In this scenario, the activation of growth-promoting, anti-apoptotic and pro-angiogenic signaling pathways leads to the production of downstream-effectors and active molecules as miRNAs released by tumor cells into exosomes and microvesicles.

OBJECTIVES:

We aim to develop a reliable tool for monitoring exosomic miRNAs in cell supernatants and blood sampling from patients during treatment with conventional and targeted therapies. As a first step we will investigate the specific signaling pathways engaged during the occurrence of drug resistance in sarcoma preclinical models. By means of next-generation sequencing we will identify the specific subset of exosomic miRNAs associated with pathway activation. Finally, we will validate exosomic miRNAs modulation in blood from sarcoma patients during treatment with standard and experimental therapies and we will correlate miRNA profiling with clinical outcome. Signaling pathway activation is a crucial event during drug resistance and the monitoring of exosomic miRNAs as liquid biopsies might become a valuable tool to predict drug resistance in a variety of tumors.

PRELIMINARY:

As a model we previously selected a cohort of patients affected by gastrointestinal stromal tumors (GIST) treated with standard therapies and we prospectively collected plasma samples between every stage evaluation. Moreover, plasma from patients with bone sarcomas (EWS and OS) treated accordingly to the ISG-OS2 and/or ISG/AIEOP_EWS2 is being collected prospectively. Exosomes were efficiently isolated as shown by nanoparticle tracking analysis (NTA) and biomarker identification. Exosome cargo will be mined and correlated with patient clinical response. Functional and validation studies will follow to identify reliable circulating biomarkers of drug sensitivity. Proposal supported by Italian Sarcoma Group.

SARCOMA

BROMODOMAIN BET INHIBITORS SHOULD BE PROPOSED FOR THE TREATMENT OF EWING SARCOMA PATIENTS WITH HIGH EXPRESSION OF THE INSULIN-LIKE GROWTH FACTOR 2 MRNA BINDING PROTEIN 3 (IGF2BP3).

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INTRODUCTION:

Ewing sarcoma (EWS) is a rare bone and soft tissue tumor which clinical heterogeneity mainly relies on epigenetic mechanisms, including post-transcriptional control of gene expression. Here we investigated for the first time the significance of the RNA binding protein IGF2BP3 in the regulation of EWS aggressiveness.

MATERIAL AND METHODS:

In the training set, 29 tumor samples from localized EWS were analyzed using Affymetrix GeneChip array. In the validation phase, 99 EWS were examined using qRT-PCR. Patient-derived cell lines and experimental models were used for functional studies.

RESULTS:

Univariate and multivariate analyses indicated IGF2BP3 as a potent indicator of poor prognosis. In vitro, IGF2BP3 increased anchorage-independent growth and migration of EWS cells sustaining expression of CD44, MMP9 and IGF-1R mRNAs. Accordingly, decreased sensitivity to the dual inhibitor anti-IGF-1R/IR OSI-906 was observed in IGF2BP3-knockout cells compared to controls. The bromodomain and extra-terminal domain inhibitor (BETi) JQ1 effectively decreased IGF2BP3 expression, its oncogenic targets and the capability of EWS cells to grow in anchorage-independent conditions.

CONCLUSIONS:

Overall, IGF2BP3 represents an indicator of prognosis in EWS therefore its detection may be of help to stratify patients according to risk. IGF2BP3/IGF-1R interaction provides additional criteria for a more effective administration of anti-IGF system targeted therapeutics. For patients with high expression of IGF2BP3 and inferior probability of survival, the use of BETi, such as JQ1, may be proposed for a clinical evaluation.

SARCOMA

IDENTIFICATION OF TUMOR ANTIGEN AS NOVEL TARGET FOR IMMUNOTHERAPY APPROACHES: THE SARCOMA TUMOR MODEL.

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BACKGROUND:

Pediatric and young adult sarcomas are a heterogeneous group of malignant tumors of bone and soft tissue origin. Most patients that present with localized stage are curable with surgery and/or chemotherapy; however, those with metastatic disease at diagnosis or those who experience a relapse continue to have a very poor prognosis. Immunotherapy is emerging as a plausible therapeutic modality because of the recent advances in other cancer types that may be translated to sarcoma. Building on the success of adoptive T cell therapy strategies to redirect immune-systems towards tumor using chimeric antigen receptors (CAR)-T cells, it is compelling to start the research of novel target antigens, since known antigens, including GD2 and HER2, are not consistently expressed in high percentage of sarcoma patients.

PROPOSAL:

We will investigate gene and protein/phosphoprotein profile of tumor cells derived from sarcoma patients by high throughput approaches. From this large screening, we will obtain relevant data to characterize: 1) novel targetable antigens for which design innovative immunotherapy approaches; 2) microenvironment features involved in regulating response to immunotherapy and that may be directly targeted by immunotherapy itself; 3) novel targetable signaling that could synergize with immunotherapy approaches.

RESEARCH METHODOLOGY:

total RNA will be run on high-density oligonucleotide microarray, using a combination of Knowledge-Based Filtering with Integration of Multiple Statistics Methods. Mass spectrometer protein/phosphoprotein analysis will corroborate the RNA profile. The collected data will be mandatory to select novel targets for which a specific single chain (scFv) will be screened in a library approach. The selected scFv will be used to design novel CAR molecules able to target sarcoma cell and/or microenvironment. In vitro/vivo models will be applied to investigate efficacy of the approach in the context of sarcoma model and to evaluate combinatory approaches tailored on sarcoma patients. This proposal is supported by the Italian Sarcoma Group.

SARCOMA

EXPRESSION OF PRAME IN RARE FORM OF SARCOMA: DESMOPLASTIC SMALL-ROUND-CELL TUMOR (DSRCT): A PROMISING TARGET FOR IMMUNOTHERAPY.

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DSRCT is rare, aggressive, and lethal sarcoma that usually develops in male adolescents and young adults. The prognosis for this disease is dismal with a 5-year overall survival of 15-30%. Indeed, most patients present with metastases at diagnosis, respond poorly to chemotherapy and die of disease within the first 2 years from diagnosis. Due to the rarity of this neoplasm, no large population based studies exist. The preferentially expressed antigen of melanoma (PRAME) is a cancer testis antigen expressed in many human malignancies and it is considered to be an attractive potential target for tumor immunotherapy, because of its either very low or absent expression in normal tissues. No data are available about the expression of PRAME in DSRCT patients. We evaluated the expression of this antigen, as mRNA and IHC analysis, in tumor specimens of eleven patients, diagnosed as DSRCT, for which biopsy samples were available. We found that PRAME is an antigen highly expressed on 82% of primary tissues of DSRCT patients, either at diagnosis or after treatment, independently of disease stage or type of therapy. PRAME could be a promising target for novel target therapy, vaccination or T-cell immunotherapy. Unfortunately, non-patient DSRCT derived cell lines were available to evaluate in preclinical model, the efficiency of T cells genetically modified with an artificial specific TCR directed towards the PRAME. For this poster-idea, we propose to generate patient-derived primary DSRCT cell lines and/or in vivo models patient derived xenograft (PDX) to evaluate in vitro and in vivo the best immunotherapeutic approach tailored on this subset of patients. The presence of certified Cell/Gene-Factories in our institution will let us to translate rapidly our finding in the clinic. In addition, we will explore the nature of immune cells infiltrating the tumor and the interaction between sarcoma cells and tumour microenvironment.

SARCOMA

GNAS MUTATION IN LOW-GRADE DEEPLY LOCATED MYXOID SOFT TISSUE TUMOURS.

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OBJECTIVES:

Non-distinctive low cellularity makes differential diagnosis of deep low-grade myxoid soft tissue tumours challenging on biopsy, and surgical management actually differs among these entities. Mutations of GNAS gene are reported in up to 61% of cases of intramuscular myxoma (IM). We aimed at assessing if Next Generation Sequencing (NGS) of GNAS can aid in differential diagnosis.

METHODS:

Consecutive cases of deep low-grade myxoid soft tissue tumours that underwent NGS (Iontorrent-Hot-Spot-Cancer Panel) for GNAS from May 2016 to April 2017 were retrieved. Clinical, pathological and molecular data were extracted.

RESULTS:

Fifteen patients met the selection criteria. Most of them were females (N=11), with a median age of 60 years. The most common site was the thigh (N=8). Mean size was 4.6 cm (interquartile range 3-6 cm). Neoplasm were seated "intramuscular" (N=13) or "within muscular fascia" (N=2). NGS analysis was evaluable in 14 patients. Several gene mutations, producing a constitutively activated protein R201Y, were identified in 11 cases, that where consequently diagnosed as IM. Three cases did not harbour GNAS mutations, two were diagnosed as myxofibrosarcoma, low-grade, and one as IM.

CONCLUSIONS:

NGS for GNAS can be helpful in differential diagnosis of deep low-grade myxoid soft tissue tumours as mutations can be detected in 92% of cases.