MELANOMA

EPIGENETIC DRUGS MODULATE LONG NONCODING RNAS EXPRESSION IN BRAF INHIBITOR-RESISTANT MELANOMA.

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Emergence of drug resistance is the major cause of failure of BRAF inhibitors (BRAFi) treatment in cutaneous melanoma (CM). Long noncoding RNAs (lncRNAs) represent a class of gene regulators acting at epigenetic, transcriptional and post-transcriptional level. LncRNAs have been implicated in chemoresistance through their ability to impair cell cycle arrest and apoptosis, but also to induce and modulate epithelial-mesenchymal transition, and cell adhesion-associated signaling pathways. LncRNAs interact with histone modifying complexes and/or DNA methyltransferases, being also targets of these epigenetic mediators. Furthermore, epigenetic drugs have been recently identified as modulators for lncRNAs function as well as their related targeting signals. Starting from these evidences, we asked the question whether epigenetic drugs could differentially affect the survival of BRAFi-resistant (VR) and -sensitive CM cells, investigating the mechanistic network involved, with a specific focus on the role of lncRNA. A panel of BRAFi-sensitive and VR CM cell lines was treated with the FDA-approved HDAC inhibitor vorinostat (SAHA). FACS analysis of annexin V-FITC/propidium iodide stained cells showed that SAHA cytotoxic activity was more pronounced on VR CM cells than on their parental counterparts. RNA-Seq analysis revealed that a large number of differentially expressed lncRNAs was modulated in VR CM cells treated with SAHA. Intriguingly, the expression of several VR up-regulated lncRNAs was decreased to levels similar to those observed in the matched parental cells. Functional analysis indicated these lncRNAs were statistically enriched in pathways involving cellular growth and proliferation, but also cellular assembly and organization. Though additional studies are required, epigenetic modulation of VR-associated lncRNAs promises to have significant therapeutic potential to restore BRAFi sensivity in CM, being concomitantly effective in killing VR cells as monotherapy. Based on our preliminary data, we could anticipate that the combined use of epigenetic and targeted drugs would increase therapeutic efficacy in CM patients relapsing to BRAFi.

MELANOMA

HIGH LEVEL OF TILS IS AN INDEPENDENT PREDICTOR OF NEGATIVE SLN STATUS.

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The validity of SLNB in melanoma is still debatable. Due to difficulties in health resources worldwide, efforts should be directed towards optimizing the indications for SLNB. Even if funds are not a problem, unnecessary surgeries can have undesirable side effects. The objective of this study was to determine whether intensity of infiltrating lymphocytes (TILs) is an independent predictor of sentinel lymph node (SLN) status in patients with primary cutaneous melanoma. Within the Melanoma Registry-IDI-IRCCS, 748 patients with primary cutaneous melanoma (≥ 1.00 mm of Breslow thickness) who underwent lymphatic mapping and SLNB were identified between January 1998 and December 2008. Tumor thickness ($p<0.0001$), mitotic rate ($p<0.0001$), ulceration ($p=0.002$) and TILs ($p=0.005$) were all associated with lymph node status in the univariate analysis. Of the patients with cutaneous melanoma who resulted negative for nodal metastasis, 49.7% had moderate/marked TILs versus 26.1 % among those patients who resulted positive for nodal metastasis. In the multivariate analysis, controlling for sex, age, mitotic rate, ulceration and Breslow, high levels of TILs in primary invasive melanoma was associated with a lower risk of developing SLN metastasis (OR:0.42; 95%CI. 0.20-0.89). Both mitotic rate and Breslow thickness remained statistically significant in the model. Our findings suggest that high level of TILs is an independent predictor of negative SLN status. Further research on possible biomarkers is warranted to develop a risk score to identify patients at high risk of developing regional metastasis and thus avoid unnecessary surgery.

MELANOMA

BASELINE RELATIVE EOSINOPHIL COUNT AS A PREDICTIVE BIOMARKER FOR IPILIMUMAB TREATMENT IN ADVANCED MELANOMA.

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ABSTRACT:
As diverse therapeutic options are now available for advanced melanoma patients, predictive markers that may assist treatment decision are needed. A model based on baseline serum lactate dehydrogenase (LDH), peripheral blood relative lymphocyte counts (RLC) and eosinophil counts (REC) and pattern of distant metastasis, has been recently proposed for pembrolizumab-treated patients. Here, we applied this model to advanced melanoma patients receiving chemotherapy (n = 116) or anti- CTLA-4 therapy (n = 128). Visceral involvement, LDH and RLC were associated with prognosis regardless of treatment. Instead, when compared to chemotherapy-treated patients with REC < 1.5%, those with REC ≥ 1.5% had improved overall survival when receiving anti-CTLA-4 [Hazard Ratio (HR) = 0.56 (0.4–0.93)] but not chemotherapy [HR = 1.13, (0.74–1.74)], and the treatment-by-REC interaction was significant for both overall (p = 0.04) and progression free survival (p = 0.009). These results indicate baseline REC ≥ 1.5% as a candidate predictive biomarker for benefit from anti-CTLA-4. Further studies are needed to confirm these findings in patients receiving immune-modulating agents.

MELANOMA

VALIDATION OF CUSTOM PANELS FOR DETECTING GERMLINE AND SOMATIC VARIATIONS IN MELANOMA: THE QUALITY CONTROL ITALIAN MELANOMA INTERGROUP (IMI) CARDS EXPERIENCE.

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INTRODUCTION:
Next-generation sequencing (NGS) methods for predisposing cancer testing have been rapidly adopted by clinical laboratories. However, no sufficient guidelines for implementing NGS test in melanoma clinical practice are established in Italy. Aim of this study was to establish analytical validation best practice guidelines for NGS gene panel testing of melanoma germline and somatic variants and develop quality controls to be adopted within IMI. The two promoting groups had passed external EMQN quality controls for germline and somatic NGS testing.

MATERIALS AND METHODS:
Two custom designed panels covering melanoma susceptibility genes (27 genes) and somatic clinically actionable mutations (25 genes) had been previously designed, and validated (using Thermo Fisher platforms) by the participating (Genoa and Sassari) groups. Five germline and six somatic DNAs were extracted from blood and FFPE tumor, respectively; samples were exchanged and blindly sequenced, for a total of 22 samples analysed from each group. Confirmation with Sanger sequencing was performed.

RESULTS:
Despite different coverage depending on the platform used and pipeline of analysis, the results were highly concordant after confirmation. Determining requirements for minimal depth of coverage, positive percentage agreement, and positive predictive value for each variant type is under evaluation. For germline variants, we already started providing NGS-based reports back to the patients for a more accurate classification of all susceptibility genes involved. For somatic mutations a consensus on variants to be reported back to the patients was not reached yet since the clinical relevance of most of them is poorly known.

CONCLUSIONS:
These target panels represent a relevant, highly scalable, and robust tool that is easy to implement and can be fully adapted to daily clinical practice in determining melanoma actionable gene mutations. The samples proved to be good reference material for evaluation of this and potentially other, more extended, NGS assay performance.

MELANOMA

DERMOSCOPIC PATTERN OF NEVI AND MELANOMAS IN PATIENTS CARRIERS OF MITF E318K GERMLINE MUTATION.

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INTRODUCTION:
a rare germline functional variant in the microphtalmia-associated transcription factor (MITF) gene, encoding a SUMOylation deficient E318K-mutated protein, has recently been identified as a medium-penetrance melanoma susceptibility gene. The number of detected variants is increasing following inclusion of this variant in next generation sequencing (NGS) panels currently used in diagnostics. Conflicting data are reported on the association of this variant with other cancers or melanoma histological subtypes. The aim of this study was to evaluate the phenotypic characterization of nevi and melanomas in the E318K mutation carriers.

MATERIALS AND METHODS:
371 familiar and 1257 sporadic cases were analysed for MITF exon 10 by DHPLC, Sanger seqeucning or NGS. For carriers of MITF E318K , histological examinations and videodermoscopic images of nevi and melanomas were recovered.

RESULTS:
26 patients carried the E318K variant (1.6%). Not all of them had undergone dermoscopy before removing skin lesions, since the clinical examinations were strongly suggestive. CDKN2A mutation carriers (3/26) were excluded. Histological examinations and dermoscopic images have been recovered for 5 patients. Of them, 2 patients developed one melanoma, 2 multiple melanomas and 1 did not develop melanoma but had inherited the mutation from affected relatives. Dermoscopically, pigmented melanocytic nevi had reticular pattern and mixed patterns (reticular-globular/reticular-homogeneous/globular-homogeneous). Pigmented melanomas and displastic nevi showed reticular pattern with peripheral foci of hyperpigmentation or atypical pigment network. Moreover, 1 of the 5 E318K carriers removed hypomelanotic/amelanotic melanomas, which are also difficult to diagnose appearing as pink-reddisk macules/papules with peripheral light brown pigmentations.

CONCLUSIONS:
our data confirm previous studies about the association of the E318K variant with the dermoscopic reticular pattern and with the hypomelanotic/amelanotic clinical variant of melanoma. Since these melanomas lack specific dermoscopic criteria and may simulate dysplastic nevi ("Nevus-like melanomas"), they are difficult to diagnose.

MELANOMA

A META-ANALYSIS OF NEVUS-ASSOCIATED MELANOMA: PREVALENCE AND PRACTICAL IMPLICATIONS.

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BACKGROUND:
Nevus-associated melanoma (NAM) is defined by the co-existence of nevus components and melanoma features on histopathological examination; on the opposite, de novo melanoma is not associated with a pre-existing nevus. The reported prevalence of NAM varies substantially in literature.

AIM AND METHODS:
We performed a systematic review and meta-analysis to determine the incidence and prevalence of NAM and to define the risk for a given melanoma to arise in association with a pre-existing nevus. We also performed sub-analyses considering, tumor thickness, and nevus-type classification. To identify eligible studies, the search was conducted in the electronic databases MEDLINE, EMBASE, and Cochrane Central Register of Controlled Trials (CENTRAL) from January 1948 through July 2016.

RESULTS:
A number of 1832 studies were retrieved, of which 347 case reports were excluded; a number of 1408 studies were also excluded on a title/abstract basis. Thus, 77 full text papers were evaluated, of which 38 observational cohort and case control studies were included in the quantitative analysis. The included studies accounted for 20126 melanomas, of which 5852 (29.1%) were nevus-associated, while the majority (70.9%) were de novo. Any given melanoma was 64% less likely to be nevus-associated than de novo (risk ratio 0.36; 95% confidence interval [CI] 0.29-0.44; P<.001; I2=99%); Sub-analysis showed that nevus-associated melanomas had a lower mean Breslow thickness than de novo melanomas (mean difference -0.39 mm; 95%CI -0.60 to -0.18; P=.0003; I2=66%). No significant differences were noted regarding the association of nevus-associated melanomas with non-dysplastic nevi or dysplastic nevi (risk ratio 0.77, 95%CI 0.49-1.20; P=.24; I2=98%).

CONCLUSIONS:
The main finding of our study is that one third of melanomas do not develop in conjunction with a pre-existing nevus. This provides further evidence that the majority of melanomas do not originate from malignant transformation of nevus cells.

MELANOMA

ACTIVITY AGAINST MELANOMA OF AN ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-1 (VEGFR-1) MONOCLONAL ANTIBODY THAT DOES NOT HAMPER LIGAND BINDING.

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VEGFR-1 is a tyrosine kinase receptor expressed in endothelium, tumor cells and monocytes/macrophages. It interacts with potent angiogenic growth factors, such as VEGF-A and PlGF. Unlike VEGF-A, which interacts also with VEGFR-2, PlGF exclusively binds to VEGFR-1. Although angiogenesis favors melanoma progression, antiangiogenic therapies, mostly based on inhibition of VEGF-A/VEGFR-2 signaling, are largely ineffective and cause severe side effects due to inhibition of physiological angiogenesis. PlGF and VEGFR-1 are frequently expressed in melanoma, promoting invasiveness and contributing to resistance to anti-VEGF-A therapy. Moreover, VEGFR-1 plays a relevant role only in pathological angiogenesis; thus, its selective targeting might produce less toxic effects. We generated an anti-VEGFR-1 mAb (D16F7) against a peptide that we had previously reported to inhibit angiogenesis and endothelial cell migration induced by VEGF-A and PlGF. D16F7 does not affect binding of VEGF-A or PlGF to VEGFR-1 but it hampers receptor homodimerization and activation, inhibiting in vitro the chemotactic response of human endothelial, myelomonocytic and melanoma cells to VEGFR-1 ligands and vasculogenic mimicry by melanoma cells. D16F7 cross-reacts with human and murine VEGFR-1. Indeed, the results of in vivo studies in a murine syngeneic model indicate that D16F7: a) inhibits angiogenesis induced by VEGF-A; b) is well tolerated; c) strongly reduces melanoma growth and spreading, increasing tumor cell apoptosis and decreasing monocytes/macrophages tumor infiltration and myeloid hematopoietic progenitors mobilization. Since the mAb acts by a non-competitive mechanism, it does not increase the amount of VEGF-A available to activate VEGFR-2, allowing VEGFR-1 to act as decoy receptor for VEGF-A and PlGF. Our results strongly suggest that a humanized D16F7 mAb, might have a therapeutic potential in metastatic melanoma as well as in other tumors or pathological conditions in which VEGFR-1 ligands, such as VEGF-A and PlGF, are involved.

MELANOMA

MELANOMA CELLS AS A MODEL TO STUDY AUTOPHAGY - RELATED PATHWAYS IN LIPID STORAGE.

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Cutaneous Melanoma (CM) is the most aggressive skin-cancer, showing high mortality at advanced stages and an increasing impact on human health; therefore new approaches against this cancer are required. Cancer stem cells (CSC) represent a key cellular subpopulation controlling biological features such as cancer progression in most cancer types; further they represent the driving force given their high growth- and metastatic-potential. By using melanospheres cell lines established from human melanoma patients, less differentiated and differentiating melanosphere-derived cells were compared to study the relationships between differentiation, lipid storage and autophagy pathways. In this study, increased lipid uptake was found in melanosphere-derived CSC vs differentiating melanosphere-derived cells, and this effect was accompanied by strong expression of two lipogenic factors, namely Sterol Regulatory Element-Binding Protein-1 (SREBP-1) and Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ). On the other hand, an inverse relation between lipid-storing phenotype and autophagy was also found, since microtubule-associated protein 1A/1B-Light Chain 3 (LC3) lipidation is reduced in melanosphere-derived CSC. To investigate the involvement of upstream autophagy regulators, Phospho-AMP activated Protein Kinase (P-AMPK) and Phospho-mammalian Target of Rapamycin (P-mTOR) were analyzed; in these models we found lower P-AMPK and higher P-mTOR expression in melanosphere-derived CSC, thus explaining, at least in part, their lower autophagic activity. In addition, confocal microscopy studies showed LC3-stained autophagosome spots co-localized with perilipin-stained lipid droplets mainly in differentiating melanosphere-derived cells, further supporting the role of autophagy in lipid droplets clearance. The reported findings, also supported by bibliometric analyses of published studies, show an inverse relationship between lipid-storing phenotype and melanoma stem cells differentiation, demonstrating an increasing role of autophagy in CM biology, therefore suggesting new potential therapeutic strategies.

MELANOMA

SCD5 AND ITS BIOPRODUCT OLEIC ACID COUNTERACT MELANOMA DISSEMINATION.

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The main biological function of the stearoyl-CoA desaturase SCD5 is the enzymatic conversion of saturated fatty acids (FAs) into mono-unsaturated FAs (MUFAs). Recently we unveiled the central role of this enzyme in melanoma progression demonstrating SCD5 downregulation in advanced melanoma and the capability of its restored expression to reduce melanoma malignancy. Specifically, SCD5 appears to increase the level of oleic acid (OA), reduce the intracellular pH, in turn blocking the release of protumoral proteins, such as SPARC. As in melanoma SPARC is one of the key factors controlling the EMT-like program, we looked for the involvement of SCD5 in reverting this process. Our in vitro experiments showed the correct modulation of some EMT-transcription factors, while a more complete reversion was obtained in vivo. In line with these findings was the SCD5-related increase of MITF, a master regulator of melanoma phenotype. Even more intriguing was the re-established sensitivity to all-trans retinoic acid in SCD5 overexpressing A375M metastatic melanoma, otherwise ineffective. Growing evidences indicate microvesiscles/exosomes as one of the way used by cancer cells to manipulate their adjacent and distant microenvironment. Our preliminary characterization of SCD5 microvesicles shows their differential cargo respect to control EXOs, including a preferential accumulation of OA, confirming the active role played by OA. Hence, future in vivo studies will be run to evaluate the EXOSCD5 capability in impairing pre-metastatic niche formation and in turn tumor spreading. Furthermore, we will consider the alternative to develop an artificial nanoparticle system (EXOOA) capable to deliver OA and its associated properties to tumor cells, hopefully preventing metastatic dissemination. Since the reduced malignancy of SCD5 expressing cells was mainly triggered by increased level of OA, a main component of the Mediterranean diet, we consider its therapeutic use as a new and side-effects-free, to be included in combination therapies.