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Phenotypic plasticity of melanocytes derived from human adult skin

DL Vidacs[1], Z Veréb[1], R Bozó[1], LB Flink[1], H Polyánka[3], BI Németh[1], S Póliska[5], BT Papp[1], M Manczinger[1], L Kemény[4] and Z Bata-Csorgo[2] *1 Department of Dermatology an Allergology, University of Medicine, Szeged, Hungary, 2 MTA-SZTE Dermatological Research Group, Eötvös Loránd Research Network, Szeged, Hungary, 3 Department of Medical Genetics, University of Szeged, Szeged, Hungary, 4 Hungarian Centre of Excellence for Molecular Medicine - University of Szeged Skin Research Group, Szeged, Hungary and 5 Department of Biochemistry and Molecular Biology, Genomic Medicine and Bioinformatics Core Facility, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*

In previous work we have shown that adult human melanocytes cultured in a defined, specific mitogen-free medium, Mel-mix became bipolar, unpigmented and highly proliferative. Furthemore, in these cells the expression of TRP-1 and c-kit disappeared and EGFR receptor and nestin expression was detected, indicating a phenotypic switch toward dedifferentiation. In the present work we used high throughput mRNA sequencing analysis to compare the dedifferentiated melanocytes to mature cells. Immunohistochemistry showed the loss of MITF and Sox-10 expression in the dedifferentiated cells and flow cytometry detected the expression of characteristic markers of mesenchymal stem cells. During dedifferentiation the expression of E-cadherin was reduced and the expression of fibronectin and oncofoetal fibronectin was elevated, confirmed by western blot. The dedifferentiated melanocytes were able to differentiate into adipogenic, osteogenic and chondrogenic phenotype given the appropriate *in vitro* environment. The extensive characterization of these cells revealed relevant similarities with undifferentiated melanoma cells, indicating that phenotype switching driven by environmental factors is a general characteristic of melanocytes that can occur independent of malignant transformation. These *in vitro* dedifferentiated melanocytes can be used to investigate the non-genetic plasticity of melanoma cells, a process that play a crucial role in metastasis, dormant cancer cell development and in therapy resistance.

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Critical role of Aquaporin-1 and telocytes in infantile hemangioma response to propranolol beta blockade

S Oucherif[1], P Kaulanjan-Checkmodine[1], S Prey[2], M Loot[2], M Jullié[2], C Leaute-Labreze[2], A Taieb[2], H Rezvani[1] and F Moisan[1] *1 Department of Life and Health Sciences, university of Bordeaux, Bordeaux, France and 2 CHU of Bordeaux, Bordeaux, France*

Propranolol is currently the first-line treatment for severe infantile hemangioma (IH), the most frequent tumor in childhood. Neither the molecular mechanism of action nor the cellular target of the drug has been identified with a strong proof of concept. We showed that propranolol antitumor activity was associated with alteration of Aquaporin 1 (AQP1), a water and ion channel involved in tumor cell migration and angiogenesis. In pathological context, elevated AQP1 is involved in the development and progression of tumors and associated with poor prognosis. We developed an IH *in vitro* model using lesional patient-derived endothelial cells (EC), pericytes (PER) and CD34^{+}/ PDGFR-α^{+} stromal cells named telocytes (TC) that we first described in IH. Thus, we investigated the role of these perivascular cells in the anti-angiogenic effect of propranolol in IH and tested AQP1 as a marker and target of the beta-blockade response in IH. Immunuhistofluorescence staining revealed a unique AQP1 expression in IH-TC compared to normal dermis. IH-TC maintain high in vitro AQP1 protein levels compared to foreskin control-TC. We performed a Matrigel based tube formation assay including IH-EC, IH-PER and IH-TC, which highlights migration and cell-cell interaction alterations. This 3D model has a unique response to low doses of propranolol. Indeed 3 µM of propranolol highly decreased IH angiogenesis in our in vitro model and this effect is abolished when control foreskin-TC are used instead of IH-TC. Interestingly, the knockdown of AQP1 in IH-TC shows the same anti-angiogenic effect as propranolol and the combination is not additive. Moreover, we show that this beta-blockade downregulates AQP1 in patient derived telocytes. All together, these data suggest that IH sensitivity to propranolol rely at least in part to a cross talk between lesional vascular cells and stromal telocytes and reveal a critical role of AQP1 in this antitumor response.

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Neurotrophin receptors: conflicting roles in cutaneous squamous cell carcinoma

M Quadri[1], N Tiso[4], F Musmeci[2], M Morasso[3], R Lotti[1], L Campanini[1], A Marconi[1], C Pincelli[1] and E Palazzo[1] *1 University of Modena, Modena, Italy, 2 Cell Dynamicas, Bologna, Italy, 3 NIAMS, NIH, Bethesda, MD and 4 University of Padua, Padua, Italy*

Cutaneous squamous cell carcinoma (cSCC) is the second most frequent form of skin cancer showing a rapidly increasing incidence worldwide. NTs mediate their effects through the common neurotrophin receptor CD271 and the tyrosine kinases family of receptors (Trks). In the skin, CD271 is implicated in the switch between stem and early progenitors, while Trk receptors mediate cell survival. However, the precise role of NT receptors in cSCC is still to be defined. Gene and protein expression analysis revealed that CD271 overexpression, while reducing SCC spheroid growth and increasing apoptosis, down-regulates TrkA and Survivin, both required for keratinocyte stemness. Conversely, CD271 silencing upregulates TrkA, as well as CXCL8, which is associated with cancer invasion. Moreover, CD271 silencing increases viability and aggressiveness of cSCC, as further demonstrated by the cSCC zebrafish model By using the novel flow-based W8^{TM} machine, we show that CD271 overexpression significantly increases mass density, reduces their weight and diameter. RNAseq analysis of CD271 overexpressing spheroids show a major fold-enrichment in cell differentiation and keratinization genes. CD271 overexpressing spheroids display low TrkA and Ki67 expression, while the expression of Keratin 1 is increased. TrkA/Fc chimera decreases cell viability and SCC spheroid invasion area, confirming the dependence of SCC cells from the autocrine NTs. K252, that blocks Trk phosphorylation, reduces spheroid invasion to the same extent as chemotherapy or 5FU, the effect being upregulated by CD271 overexpression. K252 or CD271 overexpression improves the outcome of photodynamic therapy or chemotherapy, as measured by cSCC spheroid size and viability. Our data strongly indicate the opposite functions of NT receptors in cSCC, and their modulation may be a potential treatment strategy in this cancer.

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Melanoma cells conditioned-medium induces endothelial-to-mesenchymal transition of endothelial cells

D Lechevalier[1,2], V Platel[1], C Jeannière[1], Y Le Corre[2], L Martin[2] and N Clere[1] *1 MINT, UMR INSERM 1066 CNRS 6021, Angers, France and 2 Dermatology, University Hospital Angers, Angers, France*

Endothelial-to-mesenchymal transition induces CAFs formation, which promote tumor growth, immunosuppression and metastasis. It is not described in melanoma, therefore we aimed to study the ability of melanoma cells to activate EndMT of endothelial cells. Human Umbilical Vein Endothelial Cells (HUVEC) were treated with conditioned medium (CM) from SK-MEL-28 melanoma cells. Analysis of protein expression (α-SMA and vonWillebrand Factor) was conducted by Western- blot. α-SMA and vWF double-stained cells were detected by FACS. Cell functional capacities were evaluated with wound healing and cell proliferation assays. TGF-β signaling pathway was analyzed through qRT-PCR and Western blot (pSMAD/SMAD and Snail). A significant increase of α-SMA expression in HUVEC was observed after 48 hours of treatment with CM as well as a slight decrease of vWF proteic expression. These findings have been confirmed by FACS analyses: a significant increase in the percentage of double-stained cells (α-SMA/vWF) has been shown after 48 hours. After 72 hours, there was a slight decrease of vWF expression. Moreover, wound healing and cell proliferation assays exhibited enhanced migration and proliferation of endothelial cells treated with CM compared to control cells. Finally, n the presence of CM, an increase in the p-Smad/Smad ratio and Snail was observed compared to control cells while no change in *tgfβ1* gene expression was noticed. These data show that SK-MEL-28 cells induce a phenotypic modification of endothelial cells towards a mesenchymal profile according to a mechanism independent of the TGF-β pathway. The maintenance of endothelial markers suggests a partial and potentially reversible transition. Increased cell migration and proliferation indicate the likely formation of CAFs. Further work is required to identify the involved mediators in this process. These mediators may constitute, in the future, potential new therapeutic targets for metastatic melanoma.

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Role of fibroblast-MMP14 in melanoma growth

E Pach[1], J Brinckmann[2], M Rübsam[3], M Kümper[1], C Mauch[1] and P Zigrino[1] *1 Dermatology and Venereology, University of Cologne, Cologne, Germany, 2 Department of Dermatology and Institute of Virology and Cell Biology, University of Lübeck, Lübeck, Germany and 3 Max Planck Institute for Biology of Ageing, Cologne, Germany*

Matrix metalloproteinases (MMP) are crucial in remodeling the extracellular matrix during physiological and pathological processes with diverse and complex activities. Imbalances in proteolysis contribute to the pathogenesis of many skin diseases, including fibrosis and cancer. In melanoma, a strong expression of MMP14 is detected in tumor cells and peritumoral cells. Although the functional significance for this protease in melanoma cells is better understood, it is unclear which role it has in peritumoral stromal cells. In skin, *in vivo* deletion of MMP14 in fibroblasts leads to a fibrotic-like phenotype with increased dermal thickness, collagen type I content, and tissue stiffness because of loss of collagen lysis, but not synthesis. Surprisingly, melanoma growth in the fibrotic-like dermis of these mice was decreased, accompanied by reduced cellular proliferation and vascularization. Tissue stiffness in peritumoral areas lacking fibroblasts-derived MMP14, detected by atomic force microscopy, was increased in early but not late melanoma. However, peritumoral tissue in late melanoma contained more collagen but not altered cross-linkage than controls. Culture of melanoma cells on high stiffness surfaces in combination with high amounts of fibrillar collagen type I showed reduced proliferation. Comparably, the growth of melanoma spheroids in 3D systems having high fibrillar collagen type I concentrations was inhibited. In addition, the growth of melanoma in bleomycin-induced fibrotic skin lesions of control mice was reduced. In summary, we show that the expression of stromal fibroblast-MMP14 controls melanoma growth by modifying the peritumoral extracellular matrix and suggest that increased collagen accumulation is an obstacle to melanoma growth.

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Tertiary lymphoid structures correlate with better prognosis in cutaneous angiosarcoma

T Magara, M Nakamura, Y Nojiri, M Yoshimitsu, S Kano, A Matsubara, H Kato and A Morita *Nagoya City University, Nagoya, Japan*

Immune checkpoint inhibitors (ICIs), including anti-programmed death-1 (PD-1) antibodies, were recently reported to be effective against CAS. The tumor immune response in CAS is still unclear, however, and even the prognostic value of the expression of PD-L1 in CAS remains controversial. Some reports suggest that tertiary lymphoid structures (TLSs), defined as CD20-positive B cell follicles surrounded by CD3-positive T cells, are promising biomarkers that correlate with a better prognosis in several cancers, but their correlation with the prognosis in CAS is not yet established. In the present study, we investigated tumor immunity-related factors, including PD-L1 and TLSs, to clarify useful prognostic biomarkers in CAS. We examined 61 specimens from 31 patients diagnosed with CAS, and performed immunofluorescence staining for PD-L1, PD-1, CD8, CD3 and CD20. Expression of PD-L1 and infiltration of CD8 in primary lesions did not significantly correlate with the prognosis (log-rank test, p=0.678, p=0.876). The presence of TLSs was identified as clusters of CD20-positive cells surrounded by CD3-positive cells in 28 specimens from 15 cases. Only T cells in contact with B cells in the CD20-positive cell cluster expressed PD-1. Patients with at least one TLS in the primary lesion had a significantly better prognosis than those without TLSs (log-rank, p=0.0032). In multivariate analysis, only TLSs correlated with prognosis among the choice of treatment and other immunological markers (HR=0.197, 95% CI, 0.046-0.838, p=0.028). Significantly more TLSs were observed in poorly differentiated tumors compared to well-differentiated or moderately differentiated. (Fisher's exact test, p=0.048). On the basis of these findings, the presence of TLSs appears to be a valuable prognostic biomarker for patients with CAS. The presence of TLSs also suggests that CAS is an immunologically hot tumor, similar to other carcinomas treated with ICIs. Knowledge of the presence or absence of TLSs might be useful for the treatment selection of immunotherapies targeting CAS.