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P05

DIFFERENTIATION OF MELANOMA CELL LINES BY CONDITION MEDIUM AND MELANOMA ASSOCIATED FIBROBLASTS

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Aim of the study: Complexity of the tumor microenvironment significantly influences the function of tumors and contributes to the progression of cancer. Tumor associated fibroblasts are one component of the tumor microenvironment. The function of stromal fibroblasts is well documented in tumors derivatived from squamous epithelium from head and neck. Similar mechanisms can be also expected in other tumors such as melanoma.

Materials and Methods: In this study we focused on cell culture of melanoma lines under different condition and co-culture system with melanoma associated fibroblast. Expression of specific differentiation melanoma markers we studied by immunocytochemical analysis.

Results: We had two phenotypically similar melanoma lines, which were negative for typical differential melanocytic markers like HMB45, tyrosinase and melan-A/MART-1. We used melanoma associated fibroblasts and this melanoma lines in indirect culture with aim to monitor changes at the level of differentiation markers. The result was in induction expression of the differential melanocytic markers in melanoma lines, but only in short culture for 3 days. Studied lines lost this ability during prolong culture for 14 days. When we used condition medium from human embryonic cells in this system indirect culture, we were able to detect the expression of specific markers even in prolong culture for 14 day. It was interesting that the conditioned medium was able to induce uniform changes compared with melanoma associated fibroblasts.

Conclusion: The result of this study demonstrated the function of melanoma associated fibroblasts, their possible influence to the expression of differential melanocytic markers and a certain similarity to the embryonic microenvironment. The biological activities of melanoma associated fibroblasts may by crucial to keep the tumor microenvironment.

P06

EPITHELIAL-MESENCHYMAL INTERACTION IN CANCER AS POTENTIAL TARGET FOR ANTICANCER THERAPY

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Many results demonstrate that cancer cells need for their growth and spread through organism a specific microenvironment-the tumor stroma. Tumor stroma contains, except the mesenchyme, also blood vessels, which are important in nutrition of tumor cells and the inflammatory cells. We focused our research on most abundant cell component of cancer stroma on cancer-associated fibroblasts (CAF). They are producers of extracellular components, which are necessary to formation bioactive cancer microenvironment and are able to influence the biology of tumor predominantly the differentiation status of tumor cells and their migratory potential.

We have isolated CAF from malignant tumors (squamous cell carcinoma, basal cell carcinoma (BCCF), melanoma, and skin metastasis of breast cancer) and have shown that these CAF are able to influence the differentiation status of co-cultured cells from normal squamous or breast cancer epithelium. The results were compared with control experiments using normal human dermal fibroblasts, 3T3 mouse fibroblasts, and 3T3 fibroblasts influenced by the fibroblasts prepared from the basal cell carcinoma. Our results demonstrated that expression of luminal marker keratin 8 was influenced only by CAFs prepared from any tested tumors. In contrast, all tested types of fibroblasts showed a strong stimulatory effect on the expression of basal/myoepithelial marker keratin 14. Since keratin 14 is a marker of basal myoepithelial cells and keratin 8 is a marker of luminal cells, these double-positive cells can be considered for precursor cells with properties close to stem cells. Their presence in clinical samples indeed signals very poor prognosis in cancer-suffering patiens. In conclusion, our data indicate that CAFs are able to influence the phenotype of a breast cancer cell line and this effect is based on a tumor type-unspecific mechanism.

P07

YES-ASSOCIATED PROTEIN 1 (YAP1) PROMOTES MELANOMA METASTASIS

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Melanoma is a highly aggressive neoplasm that metastasizes early during progression. The genetic basis of melanoma invasion and metastasis is only partially understood. Here we show recurrent activation of the Hippo pathway as a mechanism promoting metastatic spread of melanoma.

We demonstrate focused amplifications of Yes-associated protein 1 (*YAP1*), the upstream kinase *PAK1*, and focused deletions of its negative regulators *NF2* and *LATS1* in 34.5 % of melanomas. *YAP1* protein, the transcription cofactor acting downstream in the Hippo pathway, is highly expressed in 56% of thick (≥2mm) primary melanomas, and YAP1 levels are associated with decreased overall survival (p=0.013). *In vitro*, YAP1 overexpression promotes the invasive potential of human melanoma cells, while YAP1 knock-down significantly suppresses their invasiveness.

In vivo, YAP1 overexpression strongly promotes melanoma metastasis formation in a murine xenograft model. Our study identifies the Hippo pathway as an oncogenic driver for the metastatic phenotype of melanoma.

P08

ROLE OF CPI-17 GENE IN RECONSTITUTION OF SKIN HOMEOSTASIS IN AK LESIONS

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Actinic keratoses (AK) are precancerigenous lesions which are caused in part by the carcinogenic effect of the UV genotoxic photoproducts cyclobutane pyrimidine dimmers CPD and 6-4 photoproducts (6-4PPs). Photoreactivation is a repair mechanism carried out by photolyases which specifically recognize and repair either CPDs or 6-4PPs. Beneficial effect of such enzyme into AK treatment has been recenty postulated. The aim of the study was to analyze the molecular effect of a film-forming medical device containing photolyase and UV filters in 7 AK patients using expression array approach and bioinformatics methods.

Skin recovery after treatment was confirmed in all patients by histopathological and molecular data which found overrepresentation of genes involved in cell-cell communication, cell adhesion and homeostasis. The AK response was associated to overexpression of CPI-17 gene and determined by the initial expression level of the gene (P-value=0.001). Low levels of CPI-17 were directly associated to proinflammatory genes such as TNF (P-value=0.012) and IL-1B (P-value=0.07). Gene set analysis found that skin recovery was associated to biological process involved in tissue homeostasis and cell maintenance. This study suggests a role of CPI-17 gene in restoring skin homeostasis in AK lesions.

P09

IN-SILICO PROTEIN-PROTEIN INTERACTION NETWORKS IN CO-CULTURED MELANOCYTES AND KERATINOCYTES: EVIDENCES OF AUTOPHAGY GENES INVOLVED IN MELANOGENESIS

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Individuals carrying germinal mutations in CDKN2A gene and/or non functional variants (RHC) in MC1R gene show an increased susceptibility to develop melanoma. To date, the effect of germinal CDKN2A mutation and RHC MC1R variants in skin cells has been poorly studied. Melanocyte growth and behaviour is controlled by keratinocytes control through a complex system of paracrine growth factors and cell–cell adhesion molecules which regulate the epidermal homeostasis. Thus, in-vitro studies focused exclusively on melanocytes not reflect the in-vivo conditions.

The aim of the study was to identify molecular networks associated to presence of either germline mutations in CDKN2A or RHC variants in MC1R genes which may be related with the biological impact of both genes into melanoma susceptibility.

Keratinocytes and melanocytes were obtained from two pair of siblings belonging from two familial melanoma pedigrees regarding their germinal status of both genes. After enzymatic digestions cells were co-cultured and the global RNA was analyzed by expression arrays.

Differential gene expression data (1535 transcripts deregulated in CDKN2A mutated cells and 3570 in MC1R variants carriers) was analyzed by the web-based tool SNOW.

Statistically significant networks were identified among down regulated transcripts. Overall, 24.7% of genes in CDKN2A mutants and 27.8% in MC1R variants carriers were connected in molecular networks. The network cores were genes involved in autophagyc vacuole formation (GABARAPL2, MAP1LC3A, ULK1) or co-regulators of autophagy and/or apoptosis (SMAD3, NFKB1, SQSTM1, PRKAA1, CLN3).

28.5% of upregulated transcripts in RHC MC1R cells carrying variants were in a network in which the core was composed by genes playing a role in oxidative phosphorylation and mithocondrial ribosome (GBAS, ICT1 and PRKAA1).

Our **results** suggest that variants in both genes promote autophagy deregulation in skin cell types. Also, we have identified genes involved in the cellular levels of reactive oxygen species in MC1R variant carriers.

P10

CAPTURING THE BIOLOGICAL IMPACT OF THE STATUS OF CDKN2A AND MC1R GENES IN COCULTURED HUMAN KERATINOCYTES AND MELANOCYTES: IDENTIFICATION OF DEREGULATED PATHWAYS

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Individuals carrying germline mutations in CDKN2A and/or red hair colour variants (RHC) MC1R genes show an increased risk to develop melanoma. So far, the global biological impact of germinal p.G101W CDKN2A mutation or nonfunctional MC1R variants has been poorly studied in skin cells, in addition there is no information combining genotype status of both genes.

The aim of this study was to evaluate the global effect of germinal CDKN2A mutations (p.G101W) and MC1R RHC variants in the transcriptome of primary skin cells.

Human keratinocytes and melanocytes from two pairs of siblings from two familial melanoma pedigrees were obtained. Transcriptome variation within primary keratinocytes and melanocytes cocultures was analyzed by expression array methodology. The results from the differential gene expression analysis were evaluated by functional analysis to identify biological processes and signaling pathways significantly overrepresented in the set of desregulated genes.

Overall, 1536 transcripts were deregulated in CDKN2A mutated cells, finding a downregulation of genes playing a role in Notch signaling pathway and 5 biological processes related with gene expression regulation.

Cocultures carrying MC1R variants showed 3570 transcripts deregulated. In the set of upregulated genes was found an overrepresentation of transcripts involved in oxidative stress pathways, DNA repair pathways (Mismatch repair, Nucleotide excision repair, Base excision repair and Homologous recombination) and in signaling pathways associated to neurodegenerative diseases such as Parkinson's, Alzheimer and Huntington. In contrast, downregulated genes were associated to lysosome and endocytosis pathways which are directly related with melanosome transfer from melanocytes to surrounding keratinocytes or with biological functions linked to melanin synthesis and angiogenesis.

In summary, key molecular functions and/or pathways that are deregulated due to alterations in melanoma susceptibility gene have been elucidated using a coculture system which in turn, could be involved in initiation/progression of the disease.

P11

DISTRIBUTION OF MC1R VARIANTS AMONG MELANOMA SUBTYPES: P.R163Q IS ASSOCIATED WITH LENTIGO MALIGNA MELANOMA IN A MEDITERRANEAN POPULATION

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Melanoma tumour is classified into clinico-histopathological subtypes which may be associated with different genetic and host factors. Few studies have focused on the role of MC1R gene beyond the study of melanoma risk in individuals.

The aim was to analyze whether certain MC1R variants are associated to particular melanoma subtypes with specific clinico-histopathological features.

Clinic-pathological data of primary melanoma tumours derived from 1679 patients and the germinal status of MC1R gene were included in the study.

We detected 53 MC1R variants (11 synonymous and 43 non-synonymous). Recurrent non-synonymous variants were p.V60L (29.9%), p.V92M (11.7%), p.D294H (9.4%), p.R151C (8.8%), p.R160W (6.2%), p.R163Q (4.2%) p.R142H (3.3%), p.I155T (3.8%), p.V122M (1.5%) and p.D84E (1%). Melanoma subtypes showed differences in number of total MC1R variants (P-value=0.028) and number of Red hair colour variants (P-value=0.035). Furthermore, an association between the p.R163Q variant and lentigo maligna melanoma subtype was detected under a dominant model of heritance (OR: 2.16 95%IC: 1.07-4.37; P-value=0.044). No association was found between p.R163Q and skin phototype, eye colour or skin colour indicating that the association was independently of the role of MC1R in pigmentation. No association was observed between MC1R polymorphisms and the other melanoma subtypes.

Our findings suggest that certain MC1R variants could increase the melanoma risk by means of their impact in pathways other than pigmentation and therefore be linked to specific etiopathological melanoma subtypes.

P12

PROLIFERATING PERINEAL ULCERATIONS

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Background: Rapidly proliferating squamous cell carcinoma (SCC) is a rare, but the most feared complication of hidradenitis suppurativa (HS) of the anogenital region.

Case report: A 43-year-old man presented painful proliferations on his buttocks, progressively increasing in size. The lesions affected particularly the borders of the ulcerations of the longstanding HS. Besides multiple cutaneous cysts, recurring perianal abscesses, multiple perineal sinuses, extensive scar tissue and facial acne scars, his prior medical history was unremarkable. Clinical examination revealed multiple and easily bleeding tumors. Bilateral inguinal painful lymphadenopathies were evidenced. A PET SCAN showed hyperfixation of both inguinal lymph node areas. MRI revealed subcutaneous extension of the tumors. A skin biopsy revealed medium grade squamous cell carcinoma. Despite multiple surgical interventions, the patient rapidly died of disseminated SCC carcinomatosis.

Conclusion: Rapid recognition of HS-associated SCC is crucial as the survival rate is approximately 50% at 2 years. Wide surgical resection with grafting is the only curative therapeutic option as HS-associated SCC does not respond to chemotherapy. Radiotherapy is only considered as palliative option.