The tumor stroma - friend or foe?

The Marie Curie ITN Network CAFFEIN
1st International Conference

October 22-23, 2015

Department of Biomedicine, University of Bergen
Bergen Norway

Cancer **A**ssociated **F**ibroblast
Function in tumour cell
Expansion and **In**vasion

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Department of Biomedicine, University of Bergen
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Program: Lectures, Selected Talks and Posters

Thursday, October 22

Location: Auditorium 4, Bygg for Biologiske Basalfag (BBB)
Chairperson: Donald Gullberg

Welcome address by Donald Gullberg

9.00-9.35	**Raghu Kalluri**	*(MD Anderson Cancer Center,Houston)*
	Keynote: Tumor stroma-friend or foe?	
9.35-9.45	*Discussion*	
9.45-10.20	**Arne Östman**	*(Karolinska Institute and Centre for Cancer Biomarkers)*
	Probing clinical tumor microenvironment for discovery of biomarkers and drug targets	
10.20-10.30	*Discussion*	
10.30-10.45	Coffee	
10.45-11.20	**Lars A. Akslen**	*(University of Bergen and Centre for Cancer Biomarkers)*
	Translational studies of tumor-vascular interactions	
11.20-11.30	*Discussion*	
11.30-12.05	**Helga Salvesen**	*(University of Bergen and Centre for Cancer Biomarkers)*
	Clinical implications for understanding a CANCER in CONTEXT-examples from gynecological cancers	
12.05-12.15	*Discussion*	
12.15-12.45	Lunch+Posters	

Location: Autidorium 1, Bygg for Biologiske Basalfag (BBB)
Chairperson: Taina Pihlajaniemi

12.45-13.20	**James Harper**	*(Medimmune)*
	Modelling the complex interactions in the tumour microenvironment of Pancreatic Ductal Adenocarcinoma	
13.20-13.30	*Discussion*	
13.30-14.05	**Emanuel Rognoni**	*(Centre for stem Cells & Regenerative Medicine, King's College London)*
	Fibroblasts in skin biology	
14.05-14.15	*Discussion*	
14.15-14.30	Coffee	
14.30-15.05	**Erik Sahai**	*(London Research Institute)*
	The role of CAFs in therapy failure	
15.05-15.15	*Discussion*	
15.15-15.50	**Cédric Gaggioli**	*(Institute for Research of Cancer and aging, Nice)*
	An epigenetic switch mediates constitutive pro-invasive fibroblasts activation	
15.50-16.00	*Discussion*	
16.00	Business meeting	
19.00	Dinner	(CAFFEIN member and invited speakers only)

Friday, October 23

Location: Autidorium 1, Bygg for Biologiske Basalfag (BBB)

Chairperson: Cord Brakebusch

9.00-9.35	**Jen Morton**	*(Cancer Research UK Beatson Institute)*
	Investigating the role of TGFβ signalling in mouse models of pancreatic and intestinal cancer	
9.35-9.45	*Discussion*	
9.45-10.20	**Cédric Zeltz**	*(University of Bergen)*
	α11 integrin a major collagen receptor on mesenchymal cells in wounds and tumors	
10.20-10.30	*Discussion*	
10.30-10.45	Coffee	
10.45-11.20	**Daniela Elena Costea**	*(University of Bergen)*
	In vitro reconstruction of tumor microenvironment by use of organotypic 3D models	
11.20-11.30	*Discussion*	
11.30-12.05	**Rolf K. Reed**	*(University of Bergen and Centre for Cancer Biomarkers)*
	Spheroids and interstitial fluid pressure	
12.05-12.15	*Discussion*	
12.15-12.50	**Bjørn Tore Gjertsen**	*(University of Bergen and Centre for Cancer Biomarkers)*
	Tumor-stromal interactions in blood cell cancers: biology and implication for therapy	
12.50-13.00	*Discussion*	
13.00-13.30	Lunch+Posters	

Location: Autidorium 1, Bygg for Biologiske Basalfag (BBB)

Chairperson: Hengshuo Liu

13.30-16.30	SHORT TALKS
13.30-13.50	**Ana Cavaco**
13.50-14.10	**Henriette Ertsås**
14.10-14.30	**Ram Mohan Ram Kumar**
14.30-14.50	**Mariangela Natale**
14.50-15.00	Coffee
15.10-15.30	**Guillermo Antonio Martinez Nieto**
15.30-15.50	**Anda Ströse**
15.50-16.10	**Deusdedit Tusubira**
16.10-16.30	**Inigo Martinez-Zubiaurre**
16.40	Departure

Abstracts

Evolving role of tumor stroma in Cancer progression and metastasis

Raghu Kalluri

MD ANDERSON CANCER CENTER, HOUSTON, US

Pancreatic ductal adenocarcinoma (PDAC) is associated with marked fibrosis and stromal myofibroblasts, but their functional contribution remains unknown. Transgenic mice with the ability to delete αSMA(+) myofibroblasts in pancreatic cancer were generated. Depletion starting at either noninvasive precursor (pancreatic intraepithelial neoplasia) or the PDAC stage led to invasive, undifferentiated tumors with enhanced hypoxia, epithelial-to-mesenchymal transition, and cancer stem cells, with diminished animal survival. In PDAC patients, fewer myofibroblasts in their tumors also correlated with reduced survival. Suppressed immune surveillance with increased CD4(+)Foxp3(+) Tregs was observed in myofibroblast-depleted mouse tumors. Although myofibroblast-depleted tumors did not respond to gemcitabine, anti-CTLA4 immunotherapy reversed disease acceleration and prolonged animal survival. This study underscores the need for caution in targeting carcinoma-associated fibroblasts in PDAC.

In vitro reconstruction of tumor microenvironment by use of organotypic 3D models

Daniela Elena Costea

UNIVERSITY OF BERGEN

Considerable progress has been made in attempts to create in vitro models that are more representative of tumor complexity, particularly with cells that are surviving and/or growing in three dimensions with stroma. The 3D organotypic method used for re-constructing in vitro some of the complexity of a tumor with homo- or hetero-intercellular contacts will be presented. This method implies that the tissue of interest is reconstituted in the laboratory from isolated and purified cells in a 3D structure that follows the specific spatial arrangement and architecture of the native tissue. In the case of oral mucosa, this reconstitution implies at the most simple level that oral fibroblasts are embedded into a collagen type I gel (to reconstruct the connective tissue equivalent), on top of which are seeded oral epithelial cells (to reconstruct the epithelial tissue equivalent). Several technical issues will be addressed, such as: How to grow mixed stromal-tumor complexes in appropriate ratios reflecting pathology? What type of ECM to add? What are the physico-chemical environmental characteristics of a particular pathology/cancer stage? A couple of studies will be also presented to exemplify the scientific merit of the 3D technique for understanding cancer biology.

Identification, isolation and characterization of two distinct fibroblast cell populations from the human breast

Mikkel Morsing, Marie Christine Klitgaard, Abbas Jafari, René Villadsen, Agla J. Fridriksdottir, Jiyoung Kim, Ole William Petersen, Moustapha Kassem, Lone Rønnov-Jessen

UNIVERSITY OF COPENHAGEN, DENMARK

The human breast gland is composed of an elaborate ductal-lobular epithelium. The epithelium is embedded in extensive stroma known to take part in tissue morphogenesis. Although an epithelial cellular hierarchy including stem cells, progenitor and terminally differentiated cells is being unraveled, a similar characterization of breast fibroblasts remains elusive. Here we provide evidence for the existence of two distinct human mammary fibroblast cell populations localized to the intralobular – and interlobular stroma. In situ immunohistological stainings revealed that intralobular fibroblasts are CD105high, while interlobular fibroblasts are CD105low, which allowed their isolation by flow cytometry. The two fibroblast populations are morphologically distinct and can be expanded without interconversion as revealed by a panel of markers including NG2, CD73 and CD90. To investigate the similarities and differences between these two fibroblast populations and stromal (mesenchymal) stem cells (MSC), we examined the expression of MSC surface markers in these cells using flow cytometry and observed a pattern of CD marker expression similar to MSC. In addition, only CD105high could differentiate into osteoblastic and adipogenic lineages, evidenced by quantitation of alkaline phosphatase (ALP) activity; Oil Red O staining of lipid droplets, and RT-qPCR analysis of osteogenic and adipogenic markers. Furthermore, microarray analysis of gene expression revealed differential expression of more than 300 genes in CD105high vs. CD105low cells. Based on our current data, we propose that different fibroblast cell populations exist within the human breast stroma and that intralobular-derived CD105high fibroblast cells represent a tissue resident mesenchymal stem cell-like lineage.

The effect of chemotherapeutics on tunneling nanotube communication in acute myeloid leukemia

Maria Omsland, Øystein Bruserud, Bjørn T. Gjertsen, Vibeke Andresen

UNIVERSITY OF BERGEN, NORWAY

Acute myeloid leukemia (AML) is an aggressive and heterogenous stem cell malignancy derived from the bone marrow. Its low overall survival is caused by frequent relapses after chemotherapy. Enhanced knowledge about the biological mechanisms behind the tumor-stromal response to chemotherapy may improve the understanding of relapses. We have investigated the role of the cell-to-cell communicator tunneling nanotube (TNT) in the bone marrow compartment, and examined the effects of conventional chemotherapy on this intercellular structure. Tunneling nanotube is a membranous and F-actin containing cell-to-cell communicator about 50-200 nm in diameter formed by a variety of immune cells and cancer cells. This structure can transport various cellular components like mitochondria and lysosomes and pathogens.

In this study we have investigated the presence of this communicator between AML cells and between mesenchymal stem cells (human and mouse). Treatment of OCI-AML3 with 0.1, 1.0 and 10.0 μM cytarabine (6h and 24h) down-regulated the number of TNTs. Combination with daunorubicin (100 nM) down-regulated TNTs while combination with idarubicin (45nM) did not. We observed transport of fluorescent daunorubicin in TNTs between AML cells. When daunorubicin treated cells were co-cultured with untreated cells TNT-associated transport of daunorubicin was observed in direction toward the untreated cells. We found TNT connections between stromal cells and AML cells and observed transfer of MitoTracker-labelled mitochondria from human mesenchymal stem cells to OCI-AML3 cells, suggesting that TNTs can be used to transfer complex structures or signals from the stroma to the AML cells. Results from our study suggest that TNTs made by AML cells can be used as transport and communication devices both between AML cells as well as AML cells and stroma cells. This cellular communicator might be important for the understanding of biological mechanisms behind stromal contribution of chemoresistance and relapse after intensive chemotherapy.

Decrease in Mitochondrial Respiration is Crucial in Epithelial to Mesenchymal Transition

Sissel Elisabeth Dyrstad, Deusdedit Tusubira, Gro Vatne Røsland, James B Lorens and Karl Johan Tronstad

UNIVERSITY OF BERGEN, NORWAY

Epithelial to mesenchymal transition (EMT) is a functional and phenotypic shift involving changes in cell morphology and regulatory cascades. Both EMT, as well as its reverse process MET are critical in cancer metastasis. We hypothesiszed that metabolic reprogramming is important to fulfill the phenotypic plasticity involved in EMT. Therefore we introduced TWIST or SNAI1, two regulators of EMT, in the immortalized mammary epithelial cells MCF10A and HMLE/HMLER. In the presented study we find an acute reduction in proliferation rate as well as a marked decrease in RNA content, indicating entrance into the state of cellular quiescence. In accordance with this finding, we find an upregulation of stem cell factors including Axl, CD44, PRRX1 and Akt3. We observed a reduction in energy metabolism during EMT. In line with this, we observe a decrease in mitochondrial DNA content. The metabolic signature was further associated with distinct changes in the regulation and expression of several enzymes and regulators of glycolysis and mitochondrial respiration. The expression of miR210, a microRNA that negatively regulates mitochondrial function by targeting iron-sulfur cluster proteins was increased as a result of EMT.

These findings suggest that EMT induces both stemness in MCF10A cells, and mediates a metabolic program shift with decreased mitochondrial-, as well as glycolytic ATP production. Abrogation of the regulation of EMT, metabolism and cell stemness has been linked to tumor initiation and development. This study provides new knowledge on the potentially targetable mechanisms linking cell signaling with metabolism, under the control of TWIST or SNAI1.

The functional contribution of αSMA+ Myofibroblasts in the initiation of lung cancer

Mariangela Natale, Sylvia Vong, Komal Vadnagara, Lina Carvalho, João Nuno Moreira, Valerie LeBleu and Raghu Kalluri

CENTRO DE NEUROCIENCIAS E BIOLOGIACELULAR ASSOCIACAO, PORTUGAL

Stromal myofibroblasts wield considerable influence on epithelial cells in tissue development fibrosis and cancer. Clinical data report a strong correlation between tissue fibrosis and incidence of cancer. What remain still unclear is if it's the chronic inflammation or activated fibroblasts that contribute to the emergence of cancer. Additionally, as shown for many fibrotic diseases and cancer, epigenetic modifications in the stroma promote the sustained actived state of fibroblasts, which play an important role in regulating myofibroblasts functions in tumour growth. We noted aberrant methylation of CpG island in promoter regions of selected genes, usually important tumour suppressors, in activated fibroblasts associated with different type of fibrosis and cancer. This mechanism may promote their sustained activated state and play an important role in regulating CAFs functions in tumor growth. To gain a better understanding of the functions of activated fibroblasts in various diseases, we genetically engineered a mouse in which oncogenic K-ras expression in fibroblasts is used as a surrogate for induction of their activated state. Mice that selectively express oncogenic K-rasG12D in αSMA+ mesenchymal cells develop spontaneous lung adenocarcinoma. Gene expression profiling and other biological validations demonstrate that production of several soluble factors, including HGF, is elevated in the KrasG12D αSMA+ myofibroblasts isolated from mice before the emergence of cancer. Pharmacological inhibition of HGF/c-MET axis in K-rasG12D mice at early stage of cancer emergence significantly reduced overall tumor burden, which was not evident when mice were treated at later stage of lung cancer, suggesting that other independent oncogenic pathways drive cancer progression. Our results demonstrate that lung cancer can be initiated by proliferating stromal myofibroblasts and highlight the potential importance of activated stromal fibroblasts in the emergence of lung adenocarcinoma.

RNA Sequencing reveals Tumor Necrosis Factor α Inducible Protein 6 (TNFAIP6) as a potential Single Gene Classifier of Renal Cell Carcinoma

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Introduction and aims: The recent release of a new library preparation kit has led to improvement in cDNA library quality originating from FFPE tissues. We demonstrate the feasibility of next generation sequencing (NGS) of mRNA in FFPE tissue as compared with RNAlater® stored tissues, and a potential single gene classifier in clear cell renal cell carcinoma (ccRCC).

Methods: Paired core biopsies from 16 patients with ccRCC and adjacent non-tumorous tissue were either FFPE or stored in RNAlater® (Qiagen) for up to one year. Total RNA was extracted utilizing miRNeasy FFPE kit or miRNeasy micro kit (Qiagen), respectively. The cDNA libraries were prepared using the TruSeq RNA Access Library Prep Kit® (Illumina).

Results: FFPE and the RNAlater® datasets yield similar numbers of RNA species, differentially expressed transcripts and affected pathways. The average expression of detected transcripts in both datasets correlates with R2=0.97, and the log2 fold changes of the transcripts, which are significantly altered in both datasets (n=1106), correlates with R2=0.94. A classifier model with TNFAIP6 was developed for the FFPE dataset with a specificity of 100% and sensitivity of 94%; ROC AUC=0.98; only one normal sample was misclassified due to small admixture of cancer tissue. Classifier validation in publically available Affymetrix microarray dataset GSE53757 showed TNFAIP6 up-regulation in all tumor stages with both sensitivity and specificity of 94%; ROC AUC=0.99.
Conclusions: We describe a potential single gene classifier for ccRCC. Furthermore, NGS in FFPE tissues is feasible and correlates well with RNAlater® stored tissue. Thus, our studies open up novel diagnostic possibilities on archival biopsies.

Integrin α-11 expression in carcinoma associated fibroblasts modulates oral squamous cell carcinoma behavior

Parajuli H, Sapkota D, Rajthala S, Virlan J, Lu N, Osman T, Salwa S, Emmet McCormack E, Neppelberg E, Lybak S, Liavaag PG, Johannessen AC, Gullberg D, Costea DE

UNIVERSITY OF BERGEN, NORWAY

We have previously shown that integrin α-11 is up-regulated in cancer associated fibroblasts (CAFs) in oral squamous cell carcinoma (OSCC). The current study was to investigate its role on the modulation of OSCC cell behavior. Primary fibroblasts from OSCC patients were isolated and propagated in culture. Retroviral shRNA was used to knocked-down integrin α-11 in cultured CAFs. Expression of integrin α 11 efficiently was efficiently and stably suppressed in CAFs (CAF1α11-) at both protein and mRNA levels, without altering CAFs growth or phenotype (WST1, Population doubling). CAF1α11- demonstrated a reduced ability to remodel collagen matrices, were less migratory (mtrigel transwell assay), and were less efficient in stimulating OSCC cell invasion in 3D organotypic models. Data from in vivo experiments showed a positive correlation between tongue tumor size or bioluminescence and α-11 expression in co-injected CAFs. Delayed and smaller tumor formation was also observed in a carcinogen-induced tongue tumor in a11 knocked down mice. The results of this study suggest that the expression of integrin α-11 by CAFs is able to modulate the growth and invasive behavior of OSCC cells.

Cancer-Associated Fibroblasts from lung tumors maintain their immuno-suppressive abilities after high-dose irradiation

Turid Hellevik

UNIVERSITETSSYKEHUSET NORD-NORGE, NORWAY

Accumulated evidences indicate that high-dose radiotherapy (HD-RT) trigger stronger pro-immunogenic effects than standard low-dose regimens. However, routine implementation of RT as an efficacious cancer vaccine has not been successfully achieved. Still, effects of RT on certain immuno-regulatory elements in tumors remain unknown. We have investigated effects of HD-RT on cancer-associated fibroblasts (CAFs) and their immuno-modulating functions. We unveiled a powerful immunosuppressive effect exerted by CAF on activated T-cells, which remained after a single radiation dose of 18 Gy. Relevant immuno-suppressive molecules such as PGE2, IL-6 and -10, or TGF-β were found in CAF-conditioned medium, but their secretion was unchanged after irradiation. Finally; immunogenic cell death responses from irradiated CAF were studied. Both HMGB-1 and ATP remained undetectable in the extracellular space after high-dose irradiation. Overall, lung-CAFs exert powerful immuno-suppressive effects over activated T-cells, and this effect remains unchanged after HD-RT. Importantly, CAFs do not switch on immunogenic cell death responses after exposure to HD-RT.

Analysis of RhoA in CAFs formation and tutor promoting function

Giuseppe Scieri, Cord Brakebusch

UNIVERSITY OF COPENHAGEN, DENMARK

Despite an increasing knowledge on cancer biology, few cancer cell targeting drugs have been successfully developed to date because of the genetic heterogeneity of cancers and their ability to escape treatment by undergoing further mutations. An alternative strategy is to inhibit tumor growth by targeting tumor stroma in which fibroblast are the most abundant cells. Cancer associated fibroblasts (CAFs), in contrast to normal fibroblasts, support cancer progression. CAFs can affect growth, survival and migration of tumor cells, by secretion of growth factors, cytokines, extracellular matrix (ECM) proteins and ECM modifying metalloproteinases, moreover they can affect endothelial cells promoting angiogenesis, and recruit immune cells and bone marrow derived cells, which in turn can produce tumor promoting factors.

Nearly all CAFs display an activated, myofibroblastic phenotype similar to fibroblasts during wound healing and fibrotic conditions, illustrating the description of cancer as a “wound that does not heal”.

The small Rho GTPase RhoA, which regulates among other processes stress fiber formation and cell contractility, could be crucial for the formation of CAFs, since cell contraction is crucial for the formation of myofibroblasts. In addition to the regulation of CAF formation, RhoA signaling seems to be required for the tumor promoting function of CAFs. RhoA might be therefore an interesting target for reducing CAF formation and interfering with the invasion promoting function of CAFs.

The current project directly addresses this question using in vivo and in vitro models and it will analyze the role of RhoA in formation and function of tumor promoting CAFs.

Do tumor cell exosomes trigger a CAF-like phenotype of resident fibroblasts in PDAC?

Anda Ströse, Jörg Haier

WESTFAELISCHE WILHELMS-UNIVERSITÄT MÜNSTER, GERMANY

Cancer-associated fibroblasts (CAFs) have mainly been described as tumor-promoting stromal cells with various origins, including the recruitment from other organs and reprogramming of resident fibroblasts or other cell types. It seems natural that signals from tumor cells are responsible for the establishment of a tumor-supporting stroma. Exosomes, 30-80nm-sized membrane vesicles, pose a newly described concept of paracrine cell-cell-communication. Complex signals, including functional microRNAs, can be shuttled via exosomes. Here, we study whether exosomes derived from tumor cells are involved in the reprogramming of resident normal fibroblasts (NFs).

We used an orthotopic xenograft model of human PDAC (AsPC1) in CD1/nude mice as a source for primary CAF and NF. MicroRNA-signatures have been identified with GeneChip® miRNA 4.0 microarrays and qRT-PCR. Exosomes were purified from conditioned medium using OptiPrepTM density gradient (Van Deun et al., J Extracell Vesicles, 3, 2014). Indirect co-culture and dual-pulse treatments with 5000 AsPC1-exosomes per fibroblast were applied to study exosomal or other paracrine effects on recipient cells' microRNA-expression, proliferation (percentage Ki67-positive cells) and motility (wound closure).

We identified 18 microRNAs to be upregulated in CAF compared to NF ($FC \geq 2$, ANOVA $p \leq 0.05$, FDR $p \leq 0.25$). These comprise several microRNAs with well-described functions in cancer pathology, e.g. four members of the miR-200 family, as well as several microRNAs with yet unknown functions. Interestingly, 12 out of these 18 microRNAs are also highly expressed in AsPC1-exosomes. The same microRNAs were significantly upregulated in NF by indirect co-culture with AsPC1. Treatment of NF with AsPC1-exosomes resulted in $96 \pm 28\%$ ($p \leq 0.01$, $n=4$) enhanced proliferation and $33 \pm 24\%$ ($p \leq 0.05$, $n=5$) enhanced motility in vitro.

Signals present in tumor cell exosomes are potentially capable to trigger molecular and functional changes in resident fibroblasts. These changes may be involved in the establishment of a CAF-like phenotype.

Immuno-modulatory properties of cancer-associated fibroblasts: A study in NSCLC

Laia Gorchs, Turid Hellevik, Tor B. Stuge and Inigo Martinez

UNIVERSITY OF TROMSØ

Cancer-Associated Fibroblasts (CAFs) play central roles in the regulation of cancer establishment and progression. However, the role of CAFs in regulating tumor-associated immunity remains understudied. In this work we explore associations between CAFs and lymphocytes in non-small cell lung cancer, both in tissue specimens and in functional assays in vitro. Our results show a strong donor–independent immune-suppressive effect from CAFs, including blockade of proliferative and migratory rates of activated T-cells and down-regulation of IFNg and TNFa secretion. The effects are mediated via paracrine soluble signals since comparable outcomes were obtained using CAF-derived conditioned-medium. Additionally, immune-suppressive effects are independent of Treg function as same inhibition was obtained in Treg-depleted PBMCs samples. Moreover, we have identified potential immune-suppressive molecules in CAF-supernatants including IL-6, PGE2, IL-4, and TGFβ. Selective blockade experiments of identified immuno-suppressive factors is currently under investigation. This important CAFs-driven regulatory function should be taken into consideration for successful immunotherapy strategies.

MMP13 contributes to oral tumrigenesis by enhancing malignant phenotype of partially transformed oral keratinocytes

Sapkota D, Johannessen AC, Parajuli H, Osman TA, Rajthala S, Bruland O, Ibrahim SO, Costea DE

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Although MMP13 has been reported to be up-regulated in oral premalignant lesions, it is believed to play a major role relatively in late stages (invasion and metastasis) of oral squamous cell carcinoma (OSCC) progression. The current study aimed to investigate the pro-tumorigenic roles of MMP13 in dysplastic (partially transformed) oral keratinocytes (DOK). Using pathway focused PCR array, TaqMan qRT-PCT and Western blot validation, MMP13 was found to be significantly up-regulated in DOKs, dysplastic areas in oral squamous cell carcinoma (OSCC) and OSCCs. In vitro and in vivo functional studies were carried out by retroviral mediated silencing of MMP13 expression in cell-lines derived from oral dysplastic lesion (DOK cell-line) and OSCC (SCC25 cell-line). As expected, MMP13 silencing led to suppression of tumorigenic phenotypes (colony and sphere formation abilities; and in vivo tumorigenesis in NOD/SCID mice) of SCC25 cells. However, the suppression of in vitro and in vivo tumorigenic phenotype was significantly higher in DOK cells as compared to that of SCC25 cells. Investigation of the molecular mechanisms underlying these functional effects is underway. Taken together, up-regulation of MMP13 is an earlier event in OSCC progression and it might contribute to oral tumorigenesis by enhancing malignant phenotype of partially transformed oral keratinocytes.

Microenvironment-contextual cell signaling is attenuated with age

Henriette Christie Ertsås, Mark LaBarge and James B Lorens

UNIVERSITY OF BERGEN, NORWAY

Microenvironmental cues comprising extracellular matrix (ECM) and growth factors control cellular signaling mechanisms underlying normal and pathological cellular responses. In order to probe contextually-relevant adherent cell signaling at the single cell level, we developed a microsphere cytometry approach. Cells were adhered to microspheres that display ECM-coated surfaces and then stimulated with combinations of soluble agonists/antagonists. Signaling response was measured with fluorophore-conjugated antibodies which recognize response-dependent epitopes by multiparametric flow cytometry. Using this approach we analyzed age-dependent changes in human mammary myoepithelial and luminal epithelial cells exposed to different ECM and growth factors. We found that ECM–mediated MAP kinase and PI3K pathway activation is attenuated with age. Temporal MAP kinase signaling is delayed, and PI3 kinase signaling does not reach comparable levels in ageing cells. We speculate that this attenuated response reduces oncogene-induced senescence (OIS) in the ageing cells. Human mammary epithelial cells (HMEC) from younger women transformed with mutant EGFR demonstrate higher signaling levels and reduced survival due to OIS. Conversely we observed an age-dependent increase in cell responsiveness to growth factors in normal epithelial luminal cells. We found higher surface integrin levels with age, while integrin activation and Src-signaling distinguished luminal from myoepithelial lineage. Myoepithelial cells showed reduced integrin activation with age. These results reveal changes in ECM-mediated regulation is a consequence of ageing that may diminish tumor suppression.

In vitro analysis of tumor-promoting heterogeneity of Chondrosarcoma-associated stromal fibroblasts

Ram Mohan Ram Kumar, Philomina Selvam, Walter Born, Bruno Fuchs

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Introduction: Chondrosarcoma (CS) is a rare malignant tumor of bone that produces cartilage matrix and has a high propensity to metastasise to the lung. Current knowledge on potentially malignancy-promoting biological roles of cancer associated fibroblast (CAF) in the microenvironment of CS primary tumors is limited. Here, we studied the CS malignancy-promoting capability of primary CS-associated fibroblasts (pCSAF) in vitro. We postulate that a detailed differential analysis of gene expression signatures in pCSAF with different CS malignancy-promoting capabilities will ultimately allow a classification of patients with CS into low and high risk groups. Methods and results: The present study was approved by the local ethics committee. pCSAF isolated and propagated from fresh primary tumor resections collected from 15 CS patients operated at Balgrist University Hospital were characterised by immunoflourescent staining for the fibroblast markers α-SMA and TE-7 and compared with human foreskin fibroblast as controls. Potential passaging-provoked senescence in pCSAF was monitored by β- galactosidase staining. The tumor-promoting capability of pCSAF isolated from individual tumors was examined in vitro by assessing the colony formation-promoting activity of pCSAF conditioned medium on CS tumour cells (105KC, JJ012) cultured in soft agar. Impressively, different tumor cell migration-stimulating activity of different pCASF isolates was observed in transwell migration assays with cells CS tumour cells seeded in the upper compartment and pCASF in the lower compartment. The sequencing of RNA isolated from pCASF with remarkably different CS tumor cell colony formation-promoting and migration inducing capabilities and the analysis of individual gene expression signatures is currently in progress. Conclusion: The remarkably different in vitro malignancy-promoting capabilities of pCSAF isolated from primary tumors of individual CS patients, likely reflected in differences in gene expression profiles, point to potentially important novel tumor stroma-derived prognostic markers and treatment targets in CS.

HIGHER Δ133p53 EXPRESSION INDICATES IMPROVED SURVIVAL IN HIGH-GRADE SEROUS OVARIAN CANCERS

Katharina Bischof, Stian Knappskog, Beryl Leirvaag, Bjørn T. Gjertsen, Helga B. Salvesen, Line Bjorge

UNIVERSITY OF BERGEN, NORWAY

Background: Mutations in TP53 are early and almost ubiquitous events in the genesis of high-grade serous ovarian carcinomas (HGSOC). While the amino-terminally truncated isoform Δ133p53 modulates gene transcription and mediation of apoptosis, the biological role of the carboxy-terminally truncated p53β and p53γ variants is still debated. However, elevated expression levels of p53 isoforms have been shown to be associated with sensitivity to chemotherapy and impact on survival.

Aims: We analyzed RNA expression of selected p53 isoforms in tumor tissues from patients with HGSOC and aimed to determine their impact on survival parameters.

Methods: This single center study included 69 patients with FIGO stage IIIC/IV HGSOC. 40 patients showed good response to standard combination therapy (defined as time to recurrence ≥ 17 months) and 29 women experienced poor response (defined as progressive disease ≤ 6 months). RNA expression of full-length p53, Δ133p53, p53β and p53γ isoforms was assessed by RT-qPCR and correlated with clinical outcome parameters.

Results: The ratio of Δ133p53 expression to full-length p53 showed independent prognostic impact on overall survival (hazard ratio = 0.418, p = 0.017, 95% CI: 0.205-0.854). Further, expression level of Δ133p53 revealed significantly improved progression-free survival (hazard ratio = 0.528, p = 0.041, 95% CI: 0.286-0.973).

Conclusions: We show that high expression of Δ133p53 - both in absolute terms as well as relative to full–length p53 - leads to improved progression free and overall survival. The role of p53 isoforms as possible predictive markers for future treatment of HGSOC has to be further examined.

Integrin α11 is Involved in Fibroblast Differentiation and Cutaneous Squamous Cell Carcinoma Development

Martinez-Nieto GA, Izzi V, Liu H, Petäistö T, Koivunen J, Ruotsalainen H, Karppinen SM, Gullberg D, Pihlajaniemi T and Heljasvaara R

OULUN YLIOPISTO, FINLAND

The relevance of the extracellular matrix in the tumor context has increased in the last years, more specifically the role of the cancer-associated fibroblasts has become remarkably studied to understand carcinogenesis and to develop new therapies against tumor initiation and progression. In this scenario, integrin a11b1, a major collagen receptor expressed by fibroblasts, has been suggested as a cancer progression modulator. Previous reports demonstrated that fibroblast-derived integrin a11 subunit promotes the growth of non-small-cell lung cancer in vivo. Moreover, this integrin was shown to be related to fibroblast activation involving the Smad pathway and TGF-b expression. We have studied WT and a11-deficient mice using the DMBA-TPA chemical skin carcinogenesis model for cutaneous squamous cell carcinoma (cSCC) development previously described by Abel et al and tumor growth has been monitored. Immunohistochemistry and immunofluorescence showed an upregulation of the integrin a11 during cSCC development. Integrin a11 deficient mice had significantly difference in the tumor number and size, and papilloma incidence. Further analyses using qPCR and immunocytochemistry in papillomas and isolated fibroblasts revealed different amount of activated fibroblasts between WT and integrin a11 KO mice. aa11 deficient mice showed different essential cell functions, such as proliferation or apoptosis, affected and tumor microenvironment was remarkably different in these mice. Considering previous reports, TGF-b molecule family expression was assessed and significant differences were found between genotypes and during the stages of tumor development. These results together suggest a role for the integrin a11 in cutaneous squamous cell carcinoma development involving fibroblast activation.

The role of cancer associated derived integrin alpha 11 in ECM remodelling and breast cancer progression

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CAF-specific proteins provide important prognostic markers and targets for anticancer drugs. Recently integrin α11 (ITGA11) emerged as a new biomarker of CAFs with an unique role in the extracellular matrix reorganization and myofibroblast differentiation. While its function is still under investigation, it is already known that this integrin acts as a receptor for interstitial collagens and may be involved in cell adhesion, migration and matrix remodelling. A previous study has shown the importance of CAF-derived integrin α11 in the tumorigenicity of cancer cells in the non-small-cell-lung carcinoma (NSCLC).
In this study, we would like to investigate the potential role of stromal integrin α11 in breast cancer. The tumor insurgence, growth and metastasis are studied in an oncogenic breast mouse tumor model WT and KO for ITGA11. The mouse tumor and metastatic samples are analyzed for co-localization studies and biomarker expression. Preliminary results show that ITGA11 is mainly expressed by stromal cells in breast cancer tissues and this expression appears to be increased during the tumor progression. Tumors are also used to isolate breast cancer cells and CAFs which are characterized and used for further in vitro studies. To investigate the role of CAF-derived ITGA11 on the cancer cell invasion and remodellling of the matrix we are using a spheroid invasion assay in a 3D system in which cancer cells are co-cultured with CAFs. Imaging and bioinformatic approches are applied to quantify the migration of each cell types, their co-localization and the distribution of collagen fibers. The first data show that ITGA11 deficiency in CAFs impairs the migration of both CAFs and cancer cells co-cultured with CAFs. A high throughtput comparative analysis will also be performed in order to identify the mechanism of action and the signaling pathway underlying the functional differences observed between the CAFs expressing or not ITGA11.

High-throughput screening (HTS) for the identification of compounds capable of interfering with astrocyte-dependent growth of glioblastoma cells

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High-throughput screening (HTS) for the identification of compounds capable of interfering with astrocyte-dependent growth of glioblastoma cells

Glioblastoma multiforme (GBM) is among the most lethal tumor types. The pathological tissue contains different tumor-associated cell types, including astrocytes, which contribute to cancer biology. Our work aims at exploring the potential role(s) of crosstalk between astrocytes and malignant cells in GBM with regard to growth and drug response of the malignant cells.

According to completed studies, GBM cell lines, as well as a panel of primary GBM cultures, have shown increased growth upon co-culture with astrocytes.

Ongoing studies are performed with the double intention of identifying the molecular details of the underlying pathogenic paracrine crosstalk and identification of small molecule inhibitors able to interfere with astrocyte-dependent GBM growth.

A HTS has been established to screen low molecular weight compounds. After a pilot screening of 1700 compounds (Prestwick + Enzo libraries), 63 molecules have been identified and are currently being validated. Of these molecules, 10 specifically inhibit GBM in co-culture and 53 only have an effect on GBM monoculture. Interestingly, temozolomide (the current standard of care for GBM) fell in the category of drugs that only inhibit GBM monoculture, suggesting a protective role of the astrocytes on the malignant cells. More libraries will be screened in upcoming studies.

Future studies will continue efforts to develop in vivo-active inhibitors of astrocyte-dependent growth. Inhibitors will also be used in chemical biology studies to help identifying molecular pathways involved in the growth-supportive effect of astrocytes.

Impact of irradiation on exosome release and content: potential implication of NRG1

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Communication between cancer cells and carcinoma-associated fibroblasts (CAFs) is implicated in tumor growth and invasion (1). These messages can be soluble molecules or vesicles, like exosomes (containing numerous proteins, lipids, and nucleic acids). Although exosomes are well studied in epithelial cell types, less is known in mesenchymal cell types, especially CAFs. In this study, the model used is an hTERT-immortalized CAF from a sporadically occurring colorectal tumor. Exosomes are highly purified by density gradient ultracentrifugation (2) and characterized by Nanoparticle Tracking Analysis. Proteome analysis revealed enrichment in membrane-associated proteins such as tetraspanins, rab-GTPases, integrins, etc. which was confirmed by western blot for CD63, CD9, beta1 integrin, etc.

Neo-adjuvant treatment of rectal cancer often includes a radiation based protocol. But irradiation of a tumor not only targets cancer cells, it affects also the other cells associated with the tumor, like CAFs. Our aim is to investigate the effect of irradiation on exosome content and release by CAFs and its reciprocal impact on cancer cell invasion, survival and metastasis.

To investigate the effect of irradiation, a single dose of 10Gy is delivered and the CAFs and their secretome are harvested at different time points after irradiation. Our results showed a radiation-dependent increase of total p53, phospho-p53 and $p21^{cip1}$ proteins with a cell-cycle arrest which correlates with an increased release of exosomes. Moreover, 10Gy irradiation differentially affects miR expression in CAFs. miR-27a-3p downregulation is potentially implicated in the increase of neuregulin-1 (NRG1) mRNA and protein expression in irradiated CAFs.

Interestingly, the presence of the transmembrane protein NRG1 was also detected in exosomes and was increased after the irradiation. Ongoing experiments will reveal the functional consequences of the increased release of NRG1 positive exosomes by irradiated CAFs. This protein can have an important role by activating the Her2/HER3 pathway in colorectal cancer cells (3).

1. De Boeck A., *et al.,* Differential secretome analysis of cancer-associated fibroblasts and bone marrow-derived precursors to identify microenvironmental regulators of colon cancer progression. Proteomics, 2013.
2. Van Deun J. *et al.*, The impact of disparate isolation methods for extracellular vesicles on downstream RNA profiling. Journal of Extracellular Vesicle, 2014
3. De Boeck A., *et al.,* Bone marrow-derived mesenchymal stem cells promote colorectal cancer progression through paracrine neuregulin 1/HER3 signalling. Gut, 2013.

Laminin-332 And Laminin-binding Integrins In Pancreatic Cancer Associated Fibroblasts Differentiation

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Introduction: Laminin is involved in tissue organization, assembling into adhesive structures, and stimulates cell migration upon proteolytic cleavage. Laminin interacts with different integrins, which trigger intracellular signaling pathways involving integrin signaling associated proteins, such as RhoGTPases. Laminin-332, is ectopically expressed in cancer cells and are reported to support metastasis.
In the cancer stroma resident cells, like fibroblasts, driven by cancer cells, differentiate into cancer-associated fibroblasts (CAF), providing a permissive tumor microenvironment.

Methods: Induction of mouse fibroblasts differentiation was performed by co-culture with the pancreatic adenocarcinoma cell line AsPc-1, treatment with conditioned medium from this cancer cell line, or TGF-β1 +/- laminin-332. The cells were seeded on 2Dfibrin gel or assembled in 3Dspheroids and monitored by Immunofluorescence or Westernblot of distinct markers, like α-Smooth Muscle Actin (α-SMA).

Results: So far, we showed that some of the available antibodies against previously reported CAF markers, are not specific. α-SMA and neural/glial antigen2 seem to be more specific markers.
Laminin-332 chains are present in human pancreatic adenocarcinoma section and expressed by AsPc-I.
After seeding cells on softer substrates such as collagen I or fibrin gels, the fibroblasts retained morphology and lower levels of α-SMA. Using this in vitro model, treatment with TGF-β1 was shown to induce fibroblast differentiation. Upon treatment with blocking compounds lebein-1 (snake venom derived protein against laminin- binding integrins) and BM2 (antibody anti-α3 laminin subunit) α-SMA expression was decreased.
Future perspectives: Using CRISPR KO technology for knocking out integrin α chains on CAF, functional
assays will be performed in order to assess the function of these receptors. Further experiments are necessary to examine whether manipulation of laminin-integrin will impair or influence CAF differentiation.

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