FONDATION
MEDIC

Consortium "Tumor-Host Interaction" supported by the MEDIC Foundation

Annual Report 2010

1. Introduction

The consortium, created in 2006 around the project groups funded by grants from the MEDIC Foundation continues to prosper. Of the 11 projects which are supported by MEDIC, 9 were in their final year and most of them have developed very favorably. Particularly satisfying has been the increasing interaction between the different groups in the consortium, several of them active in studies they might not have taken on had it not been for partnerships developed within the MEDIC consortium. The stability of the present situation, calling for a more rigorous review of the scientific activities in the consortium, led to the creation of the external Scientific Advisory Board, which has been instrumental in the decision making process that culminated in awarding the first MEDIC prize. Annual scientific and financial reports are reviewed. The consortium members continue to meet annually in Lausanne and present in symposium format the progress made and the new projects that will be or have been submitted for funding. The program of the 2010 meeting, which was somewhat perturbed by the proverbial unpredictability of winter weather, is presented in table 1. The annual reports and the annual meeting presentations provide the SAB the tools to critically follow the progress made in the studies.

Table 1 Programme of the MEDIC day 2010

A. Mariotti	Function of MFG-E8/lactadherin in cancer progression
C. Ruegg	Heterotypic interactions in the tumor microenvironment: contribution to tumor metastasis
V. Piguet	Global analysis of transcription reveals BCSC-1 as a melanoma tumor suppressor that downregulates MITF
G. Ghanem	New treatments, new markers in melanoma
L. Julien	Proangiogenic programming of $CD11b^+$ myelomonocytes in breast cancer by PlGF during hematopoietic progenitors differentiation
M. Delorenzi	Analysis of Molecular Profiles and Gene Expression in clinical samples of colon cancer
C. Sotiriou	Update on the results of the breast cancer stroma project and introduction to our new research program on breast cancer molecular heterogeneity
D. Picard	Mechanistic studies on signalling crosstalk by the estrogen receptor alpha suggest new therapeutic schemes for breast cancer
T. Petrova	Transcriptional control of colon cancer
P. Martiat	The immuno-blood and marrow environment in acute leukemia: a study of natural and induced Tregs and a transcriptomic analysis of infiltrating CD3
P.Romero	Role of microRNA in CD8 T cell functions

Productive collaborations continue to develop, justifying the decision to choose for this approach. The chosen theme "tumor-host interaction" continues to be timely and allows both a common focus in the research projects as well as significant latitude in the development of the individual research lines. One group was terminated, due to a change in direction of the research activities in the Oncology Center in Lausanne. Part of the group resurfaced in Fribourg and allowed the consortium to expand further into the cancer research activities in the region.

2. Research groups, themes and received support

Table 2 lists the research projects that are supported by the MEDIC Foundation, the project title and the total amount of annual support received.

Table 2 List of projects funded by the MEDIC foundation

C.Ruegg	Role of CYR61 in tumor progression and metastasis	CHF	169'000.-
A.Mariotti	Signaling function of Lactadherin in mammary gland carcinomas	CHF	112'120.-
D.Picard	Mechanisms and functions of unusual estrogenic signaling by the host and the environment in breast cancer	CHF	147'340.-
M.Delorenzi	Tumor expression profiling In silico modeling of tumor stroma	CHF	283'400.-
V.Piguet	Molecular pathways in melanoma progression	CHF	125'000.-
T.Petrova	Analysis of PROX1 role in colon and small cell lung cancers	CHF	176'000.-
I.Stamenkovic	Tumor-host interactions in cancer progression and metastasis	CHF	88.000.-
P.Romero	Role of microRNAs in CD8 T cell function	CHF	114’012.-
C.Sotiriou	Tumor-host interaction in breast cancer	€	360'000.-
G.Ghanem	Regulation of Ras/Raf/MEK/ERK Map kinase pathway and melanoma progression	€	153'000.-
Ph.Martiat	Leukemia-host interactions	€	229'864.-

It is relevant here to note that the total volume of MEDIC supported research conducted in Lausanne has grown and presently 6 groups profit from MEDIC support. With the expansive growth of cancer research in the Lausanne academic community this does not come as a surprise. The second biggest site is Institut Jules Bordet with 3 project groups. Geneva participates with 2 groups.

3. Research programme

Three clusters of activities can be distinguished: general aspects of tumor biology, the pathobiology of breast cancer, pathobiology of colon cancer and cancer immunotherapy.

3.1 General aspects of tumor biology

This heading puts together research lines which address questions concerning the development and behaviour of cancer cells more in general and not necessarily limited to an organ or organ system. Two research lines fall into this category: the complex interactions between a variety of cells and molecules that make up the host response to growing tumor cells and basic aspects of cell function that are disrupted in cancer cells.

1. **Heterotypic interactions is the host response to tumor cell growth**
   Principal investigator: Curzio Ruegg

Cells in normal tissues, like the skin of the intestine, are subjected to well defined stimuli originating from other surrounding microenvironment that influence and control their growth, survival and differentiation. During tumor development and progression, malignant cells modify this microenvironment, for example by attracting blood and lymphatic vessels, fibroblast and inflammatory cells. In return the tumor-modified microenvironment facilitates local tumor progression and distant metastasis formation. Microenvironmental modifications may start early during tumor progression or even precede cancer formation. Collectively, tumor microenvironmental events contribute to determine the outcome of tumor progression: tumor growth, dormancy or metastasis and resistance to therapy. This is what the Ruegg laboratory studies.

In the Ruegg laboratory, we are interested in understanding how the communication between the tumor cells and their surrounding tissue is modified during tumor growth and how this modified interaction contributes to tumor progression. More specifically we are addressing the following questions:

- Tumor microenvironment: How do cells of the microenvironment, in particular inflammatory cells, promote tumor growth and metastasis? How do therapeutic interventions modify the tumor microenvironment and how do these modifications impact tumor behavior?

- Tumor angiogenesis: how does tumor vessels modulate tumor tumor growth and metastasis? How can we therapeutically exploit tumor cell - endothelial cell interaction?

- Mechanisms of tumor metastasis: How does the cross-talk between tumor cells and the microenvironment evolve during tumor metastasis ?

- Tumors evasion from anticancer therapies. How do tumors and the microenvironment react to anticancer therapies and what are the consequences to tumor progression ?

Publications

Laurent J, Touvrey C, Botta F, Kuonen F, Ruegg C.Emerging paradigms and questions on pro-angiogenic bone marrow-derived myelomonocytic cells. Int J Dev Biol. 2011;55:527-34.

Zaric J, Joseph JM, Tercier S, Sengstag T, Ponsonnet L, Delorenzi M, Rüegg C.Identification of MAGI1 as a tumor-suppressor protein induced by cyclooxygenase-2 inhibitors in colorectal cancer cells. Oncogene. 2011 Epub ahead of print

Rüegg C, Monnier Y, Kuonen F, Imaizumi N. Radiation-induced modifications of the tumor microenvironment promote metastasis. Bull Cancer. 2011 Jun;98:47-57

Laurent J, Hull EF, Touvrey C, Kuonen F, Lan Q, Lorusso G, Doucey MA, Ciarloni L, Imaizumi N, Alghisi GC, Fagiani E, Zaman K, Stupp R, Shibuya M, Delaloye JF, Christofori G, Ruegg C. Proangiogenic factor PlGF programs CD11b(+) myelomonocytes in breast cancer during differentiation of their hematopoietic progenitors. Cancer Res. 2011;71:3781-91.

Chouaib S, Kieda C, Benlalam H, Noman MZ, Mami-Chouaib F, Rüegg C. Endothelial cells as key determinants of the tumor microenvironment: interaction with tumor cells, extracellular matrix and immune killer cells. Crit Rev Immunol. 2010;30:529-45.

Kuonen F, Touvrey C, Laurent J, Ruegg C. Fc block treatment, dead cells exclusion, and cell aggregates discrimination concur to prevent phenotypical artifacts in the analysis of subpopulations of tumor-infiltrating CD11b(+) myelomonocytic cells. Cytometry A. 2010;77:1082-90.

Sofia Vala I, Martins LR, Imaizumi N, Nunes RJ, Rino J, Kuonen F, Carvalho LM, Rüegg C, Grillo IM, Barata JT, Mareel M, Santos SC. Low doses of ionizing radiation promote tumor growth and metastasis by enhancing angiogenesis. PLoS One. 2010 Jun 21;5(6):e11222.

Imaizumi N, Monnier Y, Hegi M, Mirimanoff RO, Rüegg C. Radiotherapy suppresses angiogenesis in mice through TGF-betaRI/ALK5-dependent inhibition of endothelial cell sprouting. PLoS One. 2010 Jun 11;5(6):e11084.

Rüegg C, Alghisi GC. Vascular integrins: therapeutic and imaging targets of tumor angiogenesis. Recent Results Cancer Res. 2010;180:83-101. Review.

2. Cell cycle control functions of securin and separase

Principal investigator: Ivan Stamenkovic

Regulation of cell division is a central issue in cancer research as one of the hallmarks of cancer is uncontrolled cell growth. Part of the problem is that too many cells divide too fast, resulting in too many cells. Part of the problem is that segregation of chromosomes over the daughter cells is not well regulated, leading to chromosomal imbalances. The Stamenkovic laboratory has studied proteins involved in these processes (securin and separase) of which new functions have been identified that suggest that they also play a role in secretory processes.

Securin and separase play a key role in regulating the cell cycle by controlling sister chromatid separation during anaphase. However, a growing body of evidence suggests that in addition to regulating chromosome segregation, securin and separase display functions implicated in membrane traffic in *C. elegans* and *Drosophila*. We have shown that in mammalian cells both securin and separase associate with membranes and that depletion of either protein causes robust swelling of organelles including the *trans*-Golgi network (TGN) and endocytic vesicles in the perinuclear region. These changes are accompanied by diminished constitutive protein secretion as well as impaired receptor recycling and degradation. Unexpectedly, cells depleted of securin or separase display defective acidification of early endosomes and increased membrane recruitment of vacuolar (V-) ATPase complexes that constitute the primary proton pump that maintains and acid pH in endosomes. Maintenance of an acid pH in endosomes is essential to provide a host of proteolytic enzymes with the optimal microenvironment for their function. Disruption of appropriate acidification results in ineffective protein function as well as organelle swelling. Our findings identify a new functional role of securin and separase in the modulation of membrane traffic and protein secretion that implicates regulation of V-ATPase assembly and function.

Publications

Bacac M, Fusco C, Planche A, Santodomingo J, Demaurex N, Leemann-Zakaryan R, Provero P, Stamenkovic I. Securin and separase modulate membrane traffic by affecting endosomal acidification. Traffic. 2011;12:615-26.

3.2 The pathobiology of breast cancer

Several groups in the consortium work on breast cancer, which is the most frequently encountered type of cancer in women since 1 out of 9 will develop breast cancer and unfortunately one third of these will subsequently die from this disease. The currently used factors for predicting survival and response to treatment do not sufficiently explain why in some patients the tumors progress and in others do not or why some women respond well to therapy whereas in others the tumors continue to grow. During the last years, several prognostic predictors have been developed in breast cancer using gene expression profiling technologies. Although these predictors outperform the currently used clinico-pathologic factors, they remain suboptimal. This means that in order to get a better picture of breast cancer biology, additional elements need to be considered, such as: 1) the tumor microenvironment and 2) the disseminated and circulating tumor epithelial cells. These key

elements constitute the main research axes of this group of projects. Three research projects in the consortium focus on aspects of breast cancer.

1. **Genetic heterogeneity of breast cancer**
   Principal Investigator Christos Sotiriou

> In order to get a better picture of breast cancer biology, in this project it was decided that 1) the tumor microenvironment and 2) the disseminated and circulating tumor epithelial cells needed to be more carefully studied. The tumor microenvironment consists of elements that are contributed to the tumor by the host (including vessels, mesenchymal stromal cells and inflammatory cells) and signalling molecules which play a role in the communication between cancer cells and the host response. Circulating tumor cells have been found to constitute an integral part of the biology of cancer. Their significance is insufficiently clear, although at least a fraction of these circulating cells must be responsible for cancer metastasis.

To date, most of the molecular predictors have been derived from whole tissue consisting of tumor epithelial cells and the surrounding microenvironment. Samples, which were judged to possess insufficient tumor epithelial content, were generally excluded. However, it has now repeatedly been shown that the tumor microenvironment, or stroma, influences the growth of the tumor and its ability to progress and metastasize. Here, we aimed at getting further insight on the molecular characteristics differentiating tumor-associated stroma from normal stroma. We highlighted that the stroma clearly contributes to breast cancer progression, particularly within HER2+ tumors and potentially to non-response to chemotherapy for those patients. We believe these results may help to better identify those patients for which the cancer stroma is, at least partly, responsible for their worse prognosis, and for which the stroma should thus be specifically targeted.

During the last years, the assessment of minimal residual disease (such as circulating tumor cells, CTCs, in the peripheral blood) has emerged as a promising tool in breast cancer. Although the clinical relevance of this micrometastatic disease has been proven, these molecular characteristics of these cells remain unclear.
HER2 is a prominent therapeutic target in breast cancer, and trastuzumab, a monoclonal antibody directed against this epidermal growth factor receptor, prolongs survival in the adjuvant and metastatic setting. The expression of HER2 in primary tumors is a prerequisite for trastuzumab treatment of patients with breast cancer. What we have shown is that patients with HER2-negative tumors can have HER2-positive CTCs, which could be potential targets of trastuzumab. This hypothesis is under investigation in the prospective TREAT CTC trial.

2. **Mechanisms of functioning of the estrogen receptor**
   Principal Investigator Didier Picard

> Estrogen receptor (ER) plays an important role in breast cancer, both in terms of cancer biology and as predictor of response to therapy: ER positive tumours are likely to respond to anti-estrogen drugs (notably tamoxifen). Estrogen receptor positive breast cancers tend to develop resistance for tamoxifen, however. The Picard laboratory studies the molecular biology of the estrogen receptor against this background. Mechanisms of resistance are explored as well as possibilities to overcome this resistance.

The project comprised of several sub-projects:

1. ERα signalling crosstalk and tamoxifen resistance

Part of this project has suffered from the premature departure of a postdoc. A lot of the last results obtained set the stage for the new grant proposal. Our efforts to map all ERα binding sites in the genome when ERα is activated by cAMP (signaling crosstalk) have been pursued and are now being done as a collaboration with Jason Carroll's lab at Cancer Research UK, Cambridge, one of the premier labs in this field. We are in the process of correlating chromatin binding sites and genes regulated by ERα in response to cAMP in breast cancer cells. Comparing wild-type and tamoxifen-resistant breast cancer cells will be particularly interesting in this context.

2. miRNAs regulating ERα expression

Having shown that miR-22 represses ERα expression and estrogen responses in breast cancer cells, we continued to investigate two other miRNAs that are very unusual in that they seem to increase ERα expression. We have mapped their targets in the ERα mRNA and have explored the molecular mechanisms to some extent. More is needed, though, to wrap this up and to correlate it with some physiological or pathological condition.

3. Yeast as a screening tool for novel ERα regulators

Several candidates came out of that screen. They have all been validated in yeast, and several have now been validated in mammalian cells as well. Thus, we have at least three new regulators of ERα for which we are currently trying to identify the molecular mechanisms and physiological/pathological relevance.

4. RNAi screen in mammalian cells:

Part of the genome-wide screens to identify the factors required for ERα signaling and tamoxifen resistance in mammalian cells, as proposed in the new grant application, was just done in November 2010. More screens will be considered, but for now we have a lot to do to analyze the results and to validate some of the candidates.

5. Oxysterols and breast cancer:

We have been involved in an exciting collaboration with Marcello Maggiolini's lab at the University of Calabria in Italy on the agonistic effects of the cholesterol metabolite 25-hydroxycholesterol. In contrast to was has been published by others, we found no estrogenic (or anti-estrogenic) effect for 27-hydroxycholesterol, but could show very nicely that 25-hydroxycholesterol works as an ERα agonist. This has of course implications both for the "good" and for the "bad" roles of ERα.

Publications

Carascossa S, Dudek P, Cenni B, Briand PA, Picard D.CARM1 mediates the ligand-independent and tamoxifen-resistant activation of the estrogen receptor alpha by cAMP. Genes Dev. 2010;24:708-19.

Echeverria PC, Picard D.Molecular chaperones, essential partners of steroid hormone receptors for activity and mobility.Biochim Biophys Acta. 2010;1803:641-9.

3. New prognostic factors in breast cancer: lactadherin
Principal Investigator Agnese Mariotti

In the sera of patients with disseminated breast cancer the secreted glycoprotein MFGE8/lactadherin is present at high levels. In this project we have found that MFGE8/lactadherin increases tumorigenic potential of breast cancer cells and confers increased growth potential to normal breast epithelial cells. Our data indicate that this protein cooperates with oncogenes to promote cell transformation and increases malignant behaviour of cancer cells and favours tumour progression.

MFGE8/lactadherin is a secreted glycoprotein that is present at high levels in the sera of patients with disseminated breast cancer. We have recently demonstrated that mFGE8/lactadherin enhances the tumorigenic potential of mammary carcinoma cells and promotes in vitro growth of non-tumorigenic mammary epithelial cells. Our data indicate that this protein has a dual function: it can cooperate with oncogenes and promote cell transformation and it can increase malignancy of carcinoma cells and favour further tumor growth. We have also shown that in breast carcinomas high levels of lactadherin are associated with lack of estrogen receptor expression, and they can be found in tumors expressing ErbB2 but not in those bearing ErbB2 amplification. Our data, together with those published by another group, suggest that lactadherin function varies in different breast carcinoma subtypes. In addition, when analysing genes whose expression is correlated with lactadherin in breast carcinoma we have found that Sox10 has the highest positive correlation, suggesting a possible functional link between the two proteins and a still unsuspected role for Sox10 in breast cancer development. The project aims to clarify lactadherin function in different breast carcinoma subtypes and to gain insight in the signalling pathways downstream of it that may contribute to breast carcinoma development. We also propose to analyse Sox 10 expression in breast carcinoma by both bioinformatics and immunohistochemistry and to investigate its function in breast carcinoma cells. The research proposed here will allow us to identify those breast carcinomas whose progression is promoted by lactadherin and that thus may respond to therapeutic inhibition of lactadherin function, and to discover if Sox 10 plays a role in breast cancer.

Publications

Carrascosa C, Obula RG, Missiaglia E, Lehr HA, Delorenzi M, Frattini M, Rüegg C, Mariotti A. MFG-E8/lactadherin regulates cyclins D1/D3 expression and enhances the tumorigenic potential of mammary epithelial cells.
Oncogene. 2011 epub ahead of print

3.3 The pathobiology of colon cancer

Colon cancer is the second most frequent cancer type in the western world in females and males. In spite of the fact that it is one of the most widely studied types of cancer and that the concept of stepwise progression of molecular events has been developed based on colorectal carcinogenesis many questions around this tumor type remain unsolved and overall about 50% of the patients with this disease cannot be cured. It is therefore not surprising that in the MEDIC consortium a significant effort is directed towards contributing answers to these questions. Two research lines address colorectal cancer.

1. Molecular heterogeneity of colorectal cancer

Principal Investigator Mauro Delorenzi

In this project the molecular heterogeneity of colorectal cancer is studied in a large series of cases, with detailed clinical follow-up. The basic question asked in this project is why some colorectal carcinomas do not recur after initial treatment but others recur, metastasize and finally kill the patient. Better understanding of this heterogeneity in molecular terms could lead to new diagnostic tools (determining who needs further treatment after initial surgery) and the development of new drugs effective in colorectal cancer.

In colorectal cancer, our efforts have focused on the PETACC-3, a multicenter randomized phase III study conducted within the Pan-European trial Adjuvant Colon Cancer (PETACC) network. The trial was designed to study whether addition of irinotecan to infusional 5-FU/FA would improve disease free survival (DFS) when compared to 5-FU/FA alone as adjuvant treatment in stage II and III colon cancer patients. A tissue specimen repository as well as specimen processing and DNA/RNA extraction was set up in Lausanne. Markers by Immunohistochemistry (IHC) are being assessed in Genova and in Lausanne; genome aberrations in Leuven. Data management and statistical analysis are performed by the statistic unit of the Swiss Group of Clinical Cancer Research (SAKK) in Bern, and our group in Lausanne.

Completed work includes a study showing that activating BRAF mutations are a prognostic marker: overall survival (OS) is reduced in the population with tumors that carry a BRAF mutations, and also that tumors with activating KRAS mutations have RFS and OS that are endpoints: RFS, OS and survival after relapse (SAR). This work is unpublished, some results were presented orally at ASCO 2010. Preliminary analysis of a first (pilot) batch of gene expression profiles suggested that BRAF mutated tumors have a relatively homogeneous and identifiable profile while KRAS mutated tumors have an heterogenous profile that intermixes easily with KRAS wildtype tumors. We conclude that KRAS and BRAF activating mutations induce very different downstream gene activation in CC only marginally shorter on average. Ongoing is a larger multivariate analysis on eight simultaneously assessed molecular markers to evaluate their individual and aggregated prognostic impact in combination with classical prognostic markers (TNM etc) on three endpoints: RFS, OS and survival after relapse (SAR). This work is unpublished, some results were presented orally at ASCO 2010. Preliminary analysis of a first (pilot) batch of gene expression profiles suggested that BRAF mutated tumors have a relatively homogeneous and identifiable profile while KRAS mutated tumors have an

heterogenous profile that intermixes easily with KRAS wildtype tumors. We conclude that KRAS and BRAF activating mutations induce very different downstream gene activation in CC.

Publications

Roth AD, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, Dietrich D, Biesmans B, Bodoky G, Barone C, Aranda E, Nordlinger B, Cisar L, Labianca R, Cunningham D, Van Cutsem E, Bosman F.Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol. 2010;28:466-74.

Tejpar S, Bertagnolli M, Bosman F, Lenz HJ, Garraway L, Waldman F, Warren R, Bild A, Collins-Brennan D, Hahn H, Harkin DP, Kennedy R, Ilyas M, Morreau H, Proutski V, Swanton C, Tomlinson I, Delorenzi M, Fiocca R, Van Cutsem E, Roth A.Prognostic and predictive biomarkers in resected colon cancer: current status and future perspectives for integrating genomics into biomarker discovery. Oncologist. 2010;15:390-404.

Tejpar S, Saridaki Z, Delorenzi M, Bosman F, Roth AD. Microsatellite instability, prognosis and drug sensitivity of stage II and III colorectal cancer: more complexity to the puzzle. J Natl Cancer Inst. 2011;103:841-4

Carrascosa C, Obula RG, Missiaglia E, Lehr HA, Delorenzi M, Frattini M, Rüegg C, Mariotti A. MFG-E8/lactadherin regulates cyclins D1/D3 expression and enhances the tumorigenic potential of mammary epithelial cells. Oncogene. 2011 Aug 15. Epub ahead of print

Zaric J, Joseph JM, Tercier S, Sengstag T, Ponsonnet L, Delorenzi M, Rüegg C. Identification of MAGI1 as a tumor-suppressor protein induced by cyclooxygenase-2 inhibitors in colorectal cancer cells. Oncogene. 2012;31:48-59

2. The role of Prox1 in colon cancer progression and metastasis

Principal Investigator Tatiana Petrova

In this project one particular molecular mechanism in colorectal cancer is studied. This consists of PROX1, a molecule that has been shown to be involved in intestinal adenoma development in mouse models. The experiments conducted have shown that PROX1 is involved in the interaction between the cancer cells and the host stromal cells. Furthermore, PROX1 is closely associated with the signaling pathway involved in regulation of cell differentiation in intestinal mucosa (the Wnt pathway) and also in transformation of intestinal epithelial cells into cancer cells. Present data suggest that PROX1 is involved in progression to later stages (e.g.metastasis) rather than in the initial stages of colorectal cancer development.

We earlier reported that PROX1 expression marks the transition from benign colon adenoma to carcinoma in situ. Conditional deletion of Prox1 reduced growth of intestinal adenomas in Apc mice, whereas transgenic min/+ overexpression of

Prox1 in intestinal epithelium promoted adenoma development. Furthermore, in intestinal tumors PROX1 appeared to be a direct and dose-dependent target of the ß-catenin/TCF signalling pathway, responsible f or the neoplastic transformation. Analysis of Prox1 deficient tumors and genome-wide expression profiling of CRC cells after PROX1 suppression demonstrated that PROX1 acts as a tissue-specific regulator of cell-ECM interactions and cell polarity, and it is essential for the progression of benign adenomas towards high grade dysplasia. To better understand the mechanisms of PROX1 action in CRC, we have identified PROX1 interacting proteins using shot-gun proteomics approach and in situ proximity ligation assay. We found that PROX1 is a part of TCF/ß-catenin transcriptional complex in cultured colon cancer cells, mouse intestinal epithelial cells with activated Wnt signalling and human colon adenocarcinomas. To understand the mechanism of PROX1 action, we have interrogated the genome of colon cancer cells for PROX1, TCF4 (TCF7L2) and ß-catenin binding sites and we show that TCF4, ß-catenin and PROX1 simultaneously bind to a subset of genomic enhancers, on which PROX1 acts as a transcriptional repressor. These results suggest that PROX1 is a colon-cancer specific modifier of TCF/ß-catenin signal transduction pathway. We propose that this is one of the mechanisms by which sustained Wnt signalling, observed in the majority of colon cancers, transforms an initially normal intestinal progenitor program into a cancer-specific output, which will later contribute to unrestricted tumor growth, invasion and dissemination. To test further this hypothesis, we have studied whether PROX1 contributes to later stages of CRC, such as tumor metastasis. Using a panel of 160 CRC tumors, we found that PROX1 expression is restricted to microsatellite instable CRC (in collaboration with Dr. G. Marra and Dr.F.Bosman). Furthermore, PROX1expression was observed in metastatic CRC (in collaboration with Dr. H. Bouzourene).Suppression of PROX1 in PROX1+ SW620 cells, derived from the metastatic lymph node lesion, strongly reduced development of metastases in lung, lymph nodes and liver in an orthotopic model of CRC. In line with these results, PROX1 overexpression in PROX1-negative DLD1 CRC cells significantly enhanced the development of metastases. Surprisingly, in both cases growth of primary tumor was not affected, suggesting that PROX1 has distinct roles in adenomas vs. carcinomas. To establish whether targeting PROX1 pathway is a potentially viable clinical approach, we have studied the effects of PROX1 suppression after the establishment of primary tumor and metastases. Remarkably, while control mice developed rampant metastatic lesions, suppression of PROX1 arrested growth of metastases. Injection of tumor cells directly into blood circulation demonstrated that PROX1 does not affect tumor cell extravasation or initial lung colonization, but it promotes growth of macrometastases. Taken together, these results suggest that PROX1 plays a role beyond the regulation of the transition from benign adenoma to carcinoma *in situ* identified previously, and that PROX1-regulated transcriptional network contributes to macrometastasis. Importantly, in addition to the constitutive activation of Wnt pathway, SW480, SW620 and DLD1 cells used in our study are also mutant for KRAS. Codon 12 and 13 mutations of KRAS identify a group of patients poorly responsive to anti-EGFR therapy, currently used for the treatment of metastatic CRC. Thus, patients with metastatic microsatellite stable/PROX1 /KRAS mutant tumors, for whom only limited treatment options are currently available, may potentially benefit if suitable inhibitors of PROX1 activity or expression in CRC cells are identified.

Publications

Skog M, Bono P, Lundin M, Lundin J, Louhimo J, Linder N, Petrova TV, Andersson LC, Joensuu H, Alitalo K, Haglund CH.Expression and prognostic value of transcription factor PROX1 in colorectal cancer. Br J Cancer. 2011;105:1346-51.

3.4 The pathobiology of melanoma

1. Molecular characterisation of melanoma progression: the role of BCSC1

Principal investigator Vincent Piguet

Using state of the art molecular biology techniques the Piguet laboratory has identified a new gene in melanoma, which functions as a tumor suppressor gene: its expression is decreased in human melanoma and melanoma cell lines. When the gene is introduced in melanoma cells, the cells switch from a proliferating to a migratory behavior, which would fit with a role of BCSC in melanoma progression. The characteristics of this gene and its function are further explored in this project.

Understanding the molecular alterations involved in the development and progression of metastatic melanoma is essential for a better diagnosis and targeted therapy In this project we identified BSCS-1 as a novel tumor suppressor in melanoma using a global analysis of alternative splice variants. By using *in silico* analysis of human publicly available microarray data, qRT-PCR and Western blot techniques on human biopsies we could confirm that BCSC-1 expression is decreased in human melanoma and in melanoma cell lines. Moreover, its ectopic expression blocked tumor formation in vivo and melanoma cell proliferation in vitro. We could show that BCSC-1 downregulates MITF at the transcriptional level via its interaction with Sox 10, resulting in a switch of melanoma cells from a proliferative to a migratory phenotype. We thus identified BCSC-1 as a novel regulator of MITF. BCSC-1 could be used as a marker for melanoma progression and prognosis.

2. New prognostic markers and therapeutic approaches in melanoma

Principal investigator Ghanem Ghanem

The Ghanem laboratory studies the biology of melanoma, with the intention to identitfy markers which are prognostic (distinguish melanoma with low risk of progression from high risk melanomas) and new therapies for melanoma. A promising new marker is TYRP1, of which the biology is studied in melanoma cell lines. A promising new therapy for melanoma could be Dasatinib. The mechanisms of action of this drug are explored, in order to allow identification of patients which might benefit from Dasatinib treatment.

We ran a gene profiling study in melanoma metastases as well as a validation by mainly qPCR and IHC in a total of 101 samples. We found that TYRP1 gene expression in melanoma skin metastases correlates with both DMFS and OS and with the Breslow thickness. TYRP1 gene expression is fairly conserved during

disease progression. TYRP1 could emerge as a valuable prognostic marker, especially in melanoma patients where prognostic factors at diagnosis cannot be evaluated clinically (namely unknown or ulcerated primaries) and in metastases of thin melanomas, and as a promising target for anti-melanoma therapy.
On the other hand, we ran a screening effort to evaluate new targeted therapies in a large and well characterized panel of melanoma cell lines. We found that one of these, dasatinib, inhibits cell proliferation by inducing apoptosis in melanoma cell lines. All sensitive cells expressed high levels of cKIT, no cKIT L576P activating mutation and no NRAS or BRAF activating mutations. Dasatinib inhibits cKIT, SRC, ERK and AKT phosphorylations at very low concentrations - as low as 10^{-12} M - and synergistically inhibits the activity of both cKIT and SRC. Finally, we found that cAMP/PKA stimulation may interfere with dasatinib response but in non-physiological conditions.
Dasatinib appears, as such, as a promising agent for the treatment of a selected group of melanoma patients based on tumor tissue harbouring cKIT overexpression, no mutations of cKIT, NRAS or BRAF, and a moderate phosphorylation of PKA. This finding might be complementary to the recent unprecedented encouraging therapeutic responses obtained with PLX4032, a specific inhibitor of V600E BRAF activating mutation, in targeting wildtype BRAF. Indeed, their combination might be very helpful since it is possible that both wildtype and mutated cells coexist in a same patient.

Publications

Journe F, Boufker HI, Van Kempen L, Galibert MD, Wiedig M, Salès F, Theunis A, Nonclercq D, Frau A, Laurent G, Awada A, Ghanem G.
TYRP1 mRNA expression in melanoma metastases correlates with clinical outcome.
Br J Cancer. 2011 Nov 22;105(11):1726-32

Ghanem G, Fabrice J.Tyrosinase related protein 1 (TYRP1/gp75) in human cutaneous melanoma. Mol Oncol. 2011;5:150-5.

Herraiz C, Journé F, Abdel-Malek Z, Ghanem G, Jiménez-Cervantes C, García-Borrón JC. Signaling from the human melanocortin 1 receptor to ERK1 and ERK2 mitogen-activated protein kinases involves transactivation of cKIT.
Mol Endocrinol. 2011 ;25:138-56.

3.5 Immunotherapy of cancer

Immunotherapy of cancer has been an important research focus for several decades and remains to be so. Key questions are why the immune system responds initially to the presence of abnormal (cancer) cells in the body but fails to eliminate these cells. Conceptually this might be due to failure of the immune system to recognize the cancer cells as harmful, hence no longer attacking them. Alternatively, this may be due to failure of immune-competent cells to kill the cancer cells. Effective ways to reconstitute and reinforce the immune system in its efforts to eliminate cancer cells would constitute a major breakthrough in cancer research and treatment. Two groups are working in this area, one on melanoma as a model system with emphasis on the role of miRNAs in the regulation of the function of CD8+ T-cells and the other on leukemia, with similar approaches and productive interaction between the groups.

1. **Role of miRNA species in regulating the immune response to melanoma**
Principal investigator Pedro Romero

The Romero laboratory studies mechanisms deployed by the immune system to attack cancer cells. The ultimate goal is to develop effective immunotherapies. In this project regulation of the function of a specific set of immunecompetent cells (CD8+ T-cells) is examined. It was found that their function is at least in part regulated through micro-RNA, a new species of RNA with important general gene regulatory functions and potentially important as diagnostic tool as well as target for new therapies. The group focuses on melanoma.

In agreement with the initial experimental plan, we purified CD8+ T-cell subpopulations from healthy human donors and extracted microRNAs. Subsets comprised naïve cells, effector memory CD28+ (EM28+, 'central-memory-like'), effector memory CD28- (EM28-, pre-effector cells) and fully differentiated effectors (EMRA), ordered from less to most differentiated. Microarray analysis was performed in collaboration with the group of Ph.Martiat (Inst.Jules Bordet, Brussels) to assess the expression of 365 unique microRNAs in these different subpopulations. Results showed that in all donors, CD8+ T lymphocytes expressed a limited set of microRNAs (less than 100, with 20 being expressed at high levels). Although rather surprising, this low number is comparable with previously published work on B cells and mouse T cells. Among the well expressed microRNAs in the human CD8+ T cell subsets, we found miR-21, miR-142, miR-155 as well as 7 microRNAs of the miR-17-92 cluster. Interestingly, such a high representation of this cluster has not been described before, and suggests an important role for these microRNAs in the biology of CD8+ T cells. MicroRNA expression in antigen experienced subsets was then compared to that found in naïve cells, in order to investigate the regulation of expression that may occur during differentiation. Despite inter-donor variability, this analysis showed consistent upregulation of miR-21, miR-146a and miR-155 in antigen-experienced cells, with a clear trend towards higher expression in the most differentiated subsets. By contrast, the 17-92 cluster was downregulated in antigen experienced cells, while a preferential downregulation of the miR181 cluster was observed in the EM28+ 'central memory like' subset. Interestingly, a similar trend could be observed in MelanA specific CD8 T cell clones derived from melanoma patients, with EM 28+ clones expressing lower levels of miR181a than EM28- ones.
Similar experiments were carried out in parallel in mouse lymphocytes, using the LCMV infection model as a source of in vivo activated effector cells. The results obtained in human cells could be recapitulated in this model, indicating a conserved mechanism likely to have a physiological relevance for the function of differentiated lymphocytes.
Thus, in the first part of the project, we could show that in vivo differentiation of CD8+ T lymphocytes is associated with specific modulation of the microRNA expression pattern in both mouse and human. Next step will be the functional analysis of the regulated microRNAs. To achieve this goal, different models are currently being set up in the lab, including cell lines stably overexpressing specific micro RNAs to experimentally validate *in silico* predicted targets, as well as knock-out mice that do not express miRNA-155 or the miR17-92 cluster

Publications

Baitsch L, Baumgaertner P, Devêvre E, Raghav SK, Legat A, Barba L, Wieckowski S, Bouzourene H, Deplancke B, Romero P, Rufer N, Speiser DE. Exhaustion of tumor-specific $CD8^+$ T cells in metastases from melanoma patients.J Clin Invest. 2011 1;121:2350-60

Sierro SR, Donda A, Perret R, Guillaume P, Yagita H, Levy F, Romero P. Combination of lentivector immunization and low-dose chemotherapy or PD-1/PD-L1 blocking primes self-reactive T cells and induces anti-tumor immunity. Eur J Immunol. 2011;41:2217-28

Salaun B, Yamamoto T, Badran B, Tsunetsugu-Yokota Y, Roux A, Baitsch L, Rouas R, Fayyad-Kazan H, Baumgaertner P, Devevre E, Ramesh A, Braun M, Speiser D, Autran B, Martiat P, Appay V, Romero P. Differentiation associated regulation of microRNA expression in vivo in human CD8+ T cell subsets. J Transl Med. 2011;9:44.

Speiser DE, Schwarz K, Baumgaertner P, Manolova V, Devevre E, Sterry W, Walden P, Zippelius A, Conzett KB, Senti G, Voelter V, Cerottini JP, Guggisberg D, Willers J, Geldhof C, Romero P, Kündig T, Knuth A, Dummer R, Trefzer U, Bachmann MF. Memory and effector CD8 T-cell responses after nanoparticle vaccination of melanoma patients.J Immunother. 2010;33:848-58.

Laurent J, Speiser DE, Appay V, Touvrey C, Vicari M, Papaioannou A, Canellini G, Rimoldi D, Rufer N, Romero P, Leyvraz S, Voelter V. Impact of 3 different short-term chemotherapy regimens on lymphocyte-depletion and reconstitution in melanoma patients. J Immunother. 2010;33:723-34.

2. The role of the immune response in leukemia development

Principal investigator Philippe Martiat

Like the Romero laboratory, the Martiat laboratory studies mechanisms deployed by the immune system to attack cancer cells. The ultimate goal is to develop effective immunotherapies. In this project regulation of the function of a specific set of immunecompetent cells (regulatory T-cells) is examined. It was found that their function is at least in part regulated through micro-RNA, a new species ofRNA with important general gene regulatory functions and potentially important as diagnostic tool as well as target for new therapies. The group focuses on leukemia.

Our group focuses on the influence of tumor environment on cancer cells, using acute human leukemia (AL) and the immune environment as a model. Our working hypothesis is that not only the intrinsic characteristics of the leukemic cells but also the immune response of the host determine the evolution of the disease. Further unravelling of the immune response may ultimately lead to therapeutic applications such as immunotherapy.

We first studied the function of a subset of T cells (regulatory T cells or Tregs) in peripheral blood of normal individuals and leukemic patients. We chose to do this by characterising the expression of microRNA's (miRNA), a recently discovered RNA species that plays an important role in the regulation of gene transcription. Tregs indeed appear to have a specific pattern of expression of miRNA's. The characterisation of normal individuals has been achieved and we have started to collect samples from leukemic patients and to test some.
We also are in the process of characterising the pattern of gene expression of CD3+ T cells (using transcriptomic methods) from bone marrow and white blood cells at diagnosis and in remission after chemotherapy. This will allow us to detect patterns of gene expression related to disease outcome and response to therapy, which can be eventually used in diagnosis and follow-up of leukemia patients.
The results of our studies should give us more information about the immune leukemic environment of leukemia, and with an increased number of cases, to confirm a difference not only between patients and healthy volunteers, but also between subsets of patients (children versus adults for example) or inter-patients that could be correlated with relapse-free survival, and serve as individual biomarkers.

Publications

Salaun B, Yamamoto T, Badran B, Tsunetsugu-Yokota Y, Roux A, Baitsch L, Rouas R, Fayyad-Kazan H, Baumgaertner P, Devevre E, Ramesh A, Braun M, Speiser D, Autran B, Martiat P, Appay V, Romero P. Differentiation associated regulation of microRNA expression in vivo in human CD8+ T cell subsets.
J Transl Med. 2011 20;9:44.

Fayyad-Kazan H, Rouas R, Merimi M, El Zein N, Lewalle P, Jebbawi F, Mourtada M, Badran H, Ezzeddine M, Salaun B, Romero P, Burny A, Martiat P, Badran B. Valproate treatment of human cord blood CD4-positive effector T cells confers on them the molecular profile (microRNA signature and FOXP3 expression) of natural regulatory CD4-positive cells through inhibition of histone deacetylase.
J Biol Chem. 2010;285:20481-91.

Rouas R, Akl H, Fayyad-Kazan H, El Zein N, Badran B, Nowak B, Duvillier H, Martiat P, Lewalle P. Dendritic cells generated in clinical grade bags strongly differ in immune functionality when compared with classical DCs generated in plates.
J Immunother. 2010;33:352-63.

4. Outlook

The way the MEDIC foundation supported consortium ‘Tumor-host interaction’ continues to evolve confirms that high quality research can be supported in an approach that is not competitive in the sense of the usual research grant providing institutions (National Research Foundation, Swiss Cancer League). A high standard continues to be reached through auto-evaluation, internal review within the consortium and external peer review of new applications. The research program continues to support the development of new interactions and new research directions that the individual groups alone would not have made so easily are entertained, as the new requests for 2012 submitted in 2011 will show. External peer review of the consortium constitutes a significant effort but remains an essential step towards a scientifically valid modus operandi. The independent external Scientific Advisory Board has been created (prof. F.Lejeune, prof.G.Christofori, prof. H.Moch, prof. M.Mareel), which has evaluated the overall performance of the groups, in participating (in part) in the annual research meeting and evaluating the annual reports submitted by the groups, and of the consortium as a whole. The board members have functioned as reviewers and as jury in the MEDIC prize applications. The trustees have confirmed their satisfaction with the choices made and the structures developed and have confirmed their intention to continue to support the consortium to the extent of the possible at the present level.
The Foundation does not seek a high profile but more explicit visibility of MEDIC through its research support would be desirable. An important element is here the obligation of investigators supported by MEDIC to specifically mention MEDIC support in their publications. In addition a website is under development, allowing MEDIC member groups to remain informed as to the activities of the consortium. More importantly, the site will increase visibility of the Foundation and allow Foundation Trustee members to follow more closely the research activities deployed. Another activity has been the creation of a 'MEDIC prize’ for a particularly promising young clinician scientist, which has been awarded for the first time in 2010. A call for applications for the 2011 prize has gone out and several high quality applications have been received.

Fred T Bosman
Lausanne
November 2011