FONDATION
MEDIC

Consortium “Tumor-Host Interaction” supported by the MEDIC Foundation

Annual Report 2006-2007

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Introduction

The MEDIC Foundation has supported over the last decade a variety of biomedical research projects, largely dedicated to the fight against cancer, in the Institute Jules Bordet (Brussels), the University Medical Centre in Geneva and the University Medical Centre in Lausanne. In the latter, supported groups were in the Multidisciplinary Oncology Center (CePO) and the Swiss Institute for Experimental Cancer Research (ISREC-NCCR molecular oncology). Part of the funding was at an institutional level (notably regarding Jules Bordet and CePO). The decision was taken in 2006 to create a thematically oriented consortium, to focus this group on tumor-host interaction and to convert the allocation of available resources entirely to a project based approach. The basis for scientific validation of the submitted research projects would be the Scientific Advisory Board of the Lausanne Cancer Centre, presided by Prof. Fred Bosman. External peer review would form the basis of the review of submitted grant applications. An annual meeting of the consortium members would reinforce the interactions between consortium members and serve as a platform for strategic planning of the conducted research.

Evolution of the Consortium

The group met for the first time on September 12, 2006. In a full day scientific meeting projects were introduced and new projects were proposed. The informal setting and the social activity (dinner on the eve of the meeting) contributed to a very positive atmosphere and a general feeling that through this type of interaction the consortium could grow into an intensively collaborating group. The coordinator visited the Brussels groups in November to discuss the changes in the funding approach (as of 2008 only through time limited peer reviewed grants), had very regular interactions with the Lausanne groups and regular email contact with the Geneva groups. The group meeting in the fall of 2007 will be partly dedicated to the discussion of new projects and to future developments: how to foster high quality research with a strong thematic orientation, more open than is presently the case but avoiding the administrative charge that comes along with an entirely open call for projects. A gradual shift in responsibilities from B. Fulpius to F. Bosman was realized.

Scientific Report

Influence of breast tumor microenvironment on primary tumor growth, breast cancer sub-type and metastasis

(C. Sotiriou, Brussels; M. Delorenzi, Lausanne).

During the first year of the project, we managed to separate and isolate four different cell populations from a series of breast cancers: tumor epithelial cells (EpCAM positive), leukocytes (CD45 positive), myofibroblasts (CD10 positive) and endothelial cells. Gene expression and survival analysis was carried out using 12 publicity available microarray datasets including more than 1200 systemically untreated breast cancer patients.
The following results were obtained:

1. Breast tumor myo-fibroblast stroma cell gene expression patterns differed from those isolated from normal breast tissue. While some of the differentially expressed genes are associated with extracellular matrix formation/degradation and angiogenesis, the function of several other genes is unknown.

2. Unsupervised hierarchical clustering analysis clustered breast tumor myo-fibroblasts into four subgroups recapitulating the molecular portraits of breast cancer based on ER, HER2 status and tumor differentiation.

3. Similar to tumor expression profiling studies, breast cancer myo-fibroblasts isolated from intermediate grade tumors did not show a distinct gene expression pattern but a mixture of gene expression profiles of well and poorly differentiated tumors.

4. The breast cancer myo-fibroblasts gene expression signature showed a statistically significant association with clinical outcome: breast ER-/Her2 cancers with high expression of the stroma signature showed worse prognosis.

We conclude that breast cancer stromal cells are co-determinants of breast cancer behaviour.

Search for a breast cancer signature predictive of response to chemotherapy
(M. Delorenzi, R. Iggo).

We analyzed gene expression profiles from 102 pre neo-adjuvant treated breast cancer biopsies with known pathological complete response (PCR) status, using a novel strategy. The method is based on testing the predictive power of sets of consistently co-expressed gene modules identified in a large independent reference breast cancer gene expression database.
Nine modules were tested, which were chosen based on our previous experience with breast cancer biology. They represent the following biological determinants: "basal vs luminal tumour type", "molecular apocrine tumour type", "stroma", "hypoxia", "proliferation", "T-cell infiltration", "B-cell infiltration", "adipocytes" and "interferon signaling".

Association of the "stroma" module with resistance to chemotherapy was found in the estrogen receptor negative subset of our data and led to the definition of a predictor. The predictive value of the stroma signature in multivariable models is independent of node status, grade, size and from the molecular type of the tumour. Additional analyses revealed that it is associated with relapse free survival of patients treated with anthracyclin based chemotherapy but not in patients that did not receive any form of chemotherapy.

We conclude that the stroma signature is genuinely associated with treatment efficacy (predictive for the response to treatment).

Mechanism and functions of unusual estrogenic signaling by the host and the environment in breast cancer
(D. Picard, Geneva)

A membrane bound estrogen receptor, GPR30, mediates the stimulation of proliferation by estrogen of several cell lines representing different types of carcinomas independently of the presence of estrogen receptor α (ER α)or estrogen receptor β (ER β). To facilitate the pharmacological and structure-function analysis of GPR30, we have begun to reconstitute this signaling pathway in the budding yeast.

For many years we have been studying how ERα is activated by other signaling pathways, notably growth factors and factors that lead to elevated intracellular cAMP. We demonstrated that the activation of ERα by EGF depends on the direct phosphorylation of a particular serine residue of ERα and eventually isolated a coregulator that specifically binds this receptor form. Most recently, we have made significant progress in understanding how cAMP activates Erα. Surprisingly, while it works through PKA, it does not involve the direct phosphorylation of ERα, but rather involves the phosphorylation of another factor finally leading to the increased recruitment of a coregulator (the arginine methylase CARM1) to ERα (manuscript submitted). To investigate the functional consequences of turning ERα on with different signals, we have compared the responses by gene expression profiling with human MCF-7 breast cancer cells. This shows that different ERα activators elicit vastly different genomic responses and thus presumably physiological effects (manuscript submitted).

These results shed new light on the role of estrogens in the development of breast cancer.

Molecular mechanisms that favour metastasis formation after radiotherapy
(Curzio Rüegg, CePO, M.Delorenzi, Lausanne).

Local recurrence within a previously irradiated field is associated with increased risk of metastatic progression and poor prognosis. The clinical management of this condition is a challenge, and the underlying mechanism remains largely unknown. We have found that experimental irradiation of naïve stroma mice mediates hypoxia and the concomitant development of highly invasive phenotype in tumors which is maintained after return to a normoxic microenvironment. Stroma irradiation promotes tumor invasion and metastasis by inhibiting angiogenesis, resulting in hypoxia-driven selection of invasive and metastatic cell populations. Through gene expression profiling, genetic gain and loss of function experiments and pharmacological approaches, we identified the matricellular protein CYR61 and alphaV-integrins as proteins that co-operate to mediate invasion, metastasis and resistance to hypoxia-induced tumor cell death. A CYR61 gene expression signature consisting of 8 genes correlated with tumor hypoxia and predicted shorter relapse-free survival in human breast cancer. These results identify CYR61 and alphaV-integrins as candidate therapeutic targets in patients at risk for post-radiation recurrences and illustrate the potential impact of therapy-induced microenvironmental hypoxia in determining tumor evolution.

These results shed new light on mechanisms of tumor progression following radiotherapy and raise the question of whether tumor escape and progression, as observed during anti-angiogenic therapies, might be associated with increased tumor aggressiveness. We are currently performing experiments aimed at uncovering the cellular and molecular basis of the increased metastatic activity of CYR61 expressing cells. In addition, in collaboration with C.Sotiriou we will validate the predictive value of the CYR61 signature to predict progression after adjuvant radio/chemotherapy by measuring the expression levels of the signature genes using a quantitative RT-PCR technique established in our laboratory (multiplex PCR)..

Role of a novel tumor suppressor identified by DATAS in melanoma progression
(V. Piguet, I Stamenkovic, F. Lévy)

Resulting from a genetic screen (DATAS (Differential Analysis of Transcripts with Alternative Spicing)) performed previously, we have set up methods to validate implication of specific genes in the transitions from benign lesions to tumor. This is an important platform to evaluate function of potentially significant genes identified during the genetic screen. Using DATAS, we previously identified a total of 217 sequences that are differentially expressed in metastatic melanoma and

benign nevus. Out of several candidates that we screened this year we identified a potential novel tumor suppressor gene (BCSC-1) implicated in metastatic melanoma.

RNA expression analysis by quantitative PCR demonstrates that BCSC-1 is downregulated in all patients with stage IV disease in comparison with the expression observed in benign nevus. Ectopic expression of this gene in melanoma cell lines using a lentiviral vector decreases markedly the proliferation of these cells. In vivo experiments in collaboration with F. Lévy (Ludwig Institute) showed that B16 melanoma cells transduced with TS1 induce significantly less metastasis than control cells. Furthermore, our results demonstrate that this gene blocks the proliferation of melanoma cells in the G2/M phase of the cell cycle. Current experiments aim to further understand the function of this gene in melanoma progression. We will test in the near future the capacity of the gene to control tumor invasion versus proliferation.

We conclude that BCSC-1 might be a new tumor suppressor gene with an important role in the progression of malignant melanoma.

Efficacy of Prolyl-metasarcolyd-p-fluorophenylalanine (PSF) in human melanoma treatment
(G. Ghanem)

The work focused on the mechanism of action of PSF, to gain deeper insight on its effects both in vitro and in vivo. Our in vitro results point to a rapid and complete binding of the drug to blood cells followed by an enzymatic catalysis of the drug liberating its active metabolites at sites were there is a high proteolytic activity e.g. the tumor bed. Inhibition of proteolytic enzymes suggested that 1/3 of the binding to cell membranes is specific and involves metalloproteinases. Active metabolites were released upon competition either with melanoma cells or free proteolytic enzymes.
Identification of melanoma cell membrane proteases able to recognize PSF was carried out using 2D-gelelectrophoresis and revealed the presence of proteins compatible with enzymes known to be umportant in melanoma like MMP's, ADAMs, uPA, cathepsin B or L.
The efficacy of two PSF treatment cycles was applied to human melanoma tumor-bearing nude mice. 4x5mg over 12 days repeated 3 weeks later prevented the regrowth of the tumors with no sign of toxicity as evaluated from the body weight.
It appears therefore that mechanisms involved in invasion also act as determinants of sensitivity of melanoma cells for PSF.

In conclusion, our in vitro models allowed us to explore new therapeutic options in melanoma

Immune responses in leukemic patients
(P. Martiat)

Our work investigates defective immune responses in leukemic patients and how to circumvent these defects in autologous situations or post-allogeneic HSCT. So far, we studied leukemic-specific antigen recognition by T-cells and extended our studies to the cross talk-between antigen presenting cells (APC), T-cells, T-regulating cells (T-regs) and mesenchymal stem cells (MSC).

Our initial studies failed to demonstrate specific anti-leukemia T-cell generation in CML patients and we are currently studying the reason for this. To understand how the host environment impacts on immune responses to leukemia, we first investigated the effects of MSCs on $CD3^+$ T-cells in healthy volunteers and explored their immunoregulation mechanisms. MSCs induce a significant and dose-dependent contact-dependent reduction of T-cell proliferation whatever the stimulation

used (aspecific polyclonal activators, allostimulation). At low MSC/T-cell ration, increased T-cell proliferation was observed linked to IL-6 production. Immunosuppressive properties of MSCs thus result from a balance between inhibition and activation. Using specific Toll-like receptor (TLR)-agonists, the study of the role of TLR in MSC differentiation, proliferation and function is in progress. Furthermore, we initiated the study of MSCs collected from leukemic patients starting with the characterization of both their phenotype and function.
Presently we are carrying out
- functional studies for investigating MSC impact on anti-leukemia response
- mechanisms involved in T-reg generation and their interaction with anti-leukemic responses
- development of lentiviral vectors for gene delivery into human hematopoietic cells
- further characterization of our ovine leukemia model for studying leukemia-host interactions

- a phase I-II trial to evaluate the safety and efficacy of infusion of ex vivo-expanded MSC in 7 patients after allogeneic HSCT

These results indicate the importance of the host immune response for the evolution of leukemia and open new horizons for therapeutic intervention.

In summary

The studies conducted with MEDIC support

1. indicate that breast cancer stromal cells are co-determinants of breast cancer behaviour.
2. show that the breast cancer stroma signature is genuinely associated with treatment efficacy (predictive for the response to treatment).
3. shed new light on the role of estrogens in the development of breast cancer
4. elucidate novel mechanisms of tumor progression following radiotherapy
5. suggest that BCSC-1 might be a new tumor suppressor gene with an important role in the progression of malignant melanoma
6. explore new therapeutic options in melanoma using in vitro models
7. indicate the importance of the host immune response for the evolution of leukemia and open new horizons for therapeutic intervention.

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C.Sotiriou

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