Sunday 27 June 2010 12:45–13:45

Young Cancer Researcher's Workshop: How to be effective in applying for fellowships

32 Applying for Fellowships

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Fellowships often carry significant prestige and can provide a valuable career transition between postdoctoral and independent investigator positions. This workshop will describe some of the fellowship opportunities that are currently available in Europe. Members of fellowship interview panels will also discuss what they are looking for in candidates and provide advice on how to increase the chances of an application being successful.

Sunday 27 June 2010 13:45–14:35

Special Session: Norwegian Pink Ribbon Lecture

33 Genomic analysis of inherited breast and ovarian cancer: a model for personalized medicine

No abstract received.

Sunday 27 June 2010 14:35–16:05

Presidential Session
Presidential Session I

34 Diacylglycerol kinase contribution to mTOR-mediated breast cancer progression

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Introduction: The mammalian target of rapamycin (mTOR) is a master regulator of cell growth, proliferation and survival. The two mTOR complexes, mTORC1 and mTORC2, differ in their mTOR partners and in the substrates they phosphorylate. mTORC1 contains the protein Raptor and phosphorylates many targets, including S6 kinase, whereas mTORC2 includes Rictor and is reported to phosphorylate AKT. Rapamycin and its analogs are mTOR inhibitors with potential to control cancerous states. Several transformed cell types are rapamycin resistant. Since rapamycin and phosphatidic acid (PA) compete for mTOR binding, high PA levels and/or activity of PA generating enzymes are thought to contribute to this resistance. Diacylglycerol kinases (DGK) belong to a family of lipid kinases that phosphorylate diacylglycerol to produce PA, and are promising therapeutic targets.
Methods: To determine how DGK modulates mTOR activity and contributes to tumour progression, we used a panel of human breast cancer-derived cell lines as models in which to downmodulate DGK activity with a pharmacological inhibitor (R59949) or by RNA interference (RNAi).
Results: In these cells, rapamycin did not inhibit AKT, whose phosphorylation was maintained or increased. In contrast, R59949 decreased AKT phosphorylation and counteracted rapamycin effects. Rapamycin had no effect on cell cycle progression and R59949 alone delayed S phase entry, which was potentiated by simultaneous use of both drugs. To determine the mechanism of R59949 action, we immunoprecipitated mTOR. R59949 treatment did not affect mTOR complex composition and rapamycin affected only mTORC1 assembly; combined, the drugs strongly reduced Rictor association to mTOR.
To test the specific contribution of DGKα and ζ isoforms, we measured their levels in our breast cell panel and found no difference in DGKζ expression, but DGKα levels correlated inversely with the degree of cell line malignancy. Whereas RNAi knockdown of either isoform impaired mTORC1 activity, only DGKα depletion reduced AKT activation during cell cycling. To confirm the DGK effect on tumour progression, we generated tumours with reduced DGKα or DGKζ levels in immunocompromised mice. While DGKζ depletion had no effect, a reduction in DGKα significantly impaired tumour growth.
Conclusions: Our data suggest that DGKα-derived PA is needed for mTORC2 complex integrity and its correct function, and thus for tumour progression. DGK inhibition could be tested alone or in combination with rapamycin to design effective anti-cancer therapies.

35 Adipocyte-derived fibroblasts contribute to the desmoplastic reaction in breast cancer: a new link between breast cancer and obesity?

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In a variety of tumours such as breast carcinomas, a desmoplastic response, characterized by the presence of dense collagenous stroma comprising fibroblast-like cells, is observed and is thought to contribute to tumour progression. Peritumoural fibroblasts are composed of several subpopulations that are morphologically undistinguishable and their origins remain debated. Most of the studies have focused on the activation of fibroblasts present in the interstitium (the so-called myofibroblasts) and very little attention has been given to adipocytes, although it is obvious that in breast, early local tumour invasion results in immediate proximity of cancer cells to adipose tissue. In this study we demonstrate that tumour cells modify mature adipocytes leading to the accumulation of an activated population with morphological features of fibroblast cells. Using an original 2D system, where an insert separates the two cell populations, we show that mature adipocytes cocultivated with breast tumour cells for 3 to 8 days exhibit loss of lipid content, decrease in differentiation markers and undergo morphological and functional changes into fibroblast-like cells associated to cytoskeleton reorganization. Interestingly, this population of adipocyte-derived fibroblasts (ADF) exhibits activated phenotype with enhanced migratory, invasive and profibrotic capacities (increase of fibronectin and collagen I secretions). Finally, we report that tumour cells profoundly inhibit the adipogenesis of pre-adipocytes, suggesting a role for precursor components of the adipose tissue in the establishment of ADF cells in breast tumour stroma. Ongoing experiments are performed in our laboratory to assess the presence of ADF in human breast tumours and in mouse tumour xenografts. Our results might provide an explanation for the poor prognosis observed in localised breast cancer in obese women, since the nature of the desmoplastic reaction and the secretion pattern of the ADF might be profoundly altered in this physiopathological condition.

36 Do breast cancers arise in areas of the breast that pre-diagnostically had high mammographic density?

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Background: Percent breast density (PD) is a strong marker of susceptibility to breast cancer, but it is not known whether PD is a generalised marker of susceptibility or a more localised one with tumours arising in dense breast areas. Two previous studies have produced conflicting results.
Methods: We conducted a study nested within the intervention arm of the Age Trial, in which ~54,000 women underwent annual mammography from age 40 to 48 (thereafter every 3 years). 794 women were diagnosed with breast cancer during follow-up. Diagnostic and pre-diagnostic mammograms from 232 cases of incident breast cancer were digitised. Radiologists identified the tumour location on diagnostic films and registration techniques were used to identify the corresponding area on pre-diagnostic films. For each woman, a square grid was drawn on the pre-diagnostic film and PD of the square that contained the eventual tumour ("case" square) was compared with PD in "control" squares on the same film. "Control" squares were chosen using various methods, e.g. use of all complete (i.e. excluding incomplete squares near the edge) squares or a random selection of 3 complete squares. We performed a matched case-control analysis to investigate whether pre-diagnostic localized square-specific PD was associated with eventual tumour location. The analysis was conducted 4 times, with square-grids of length 1, 2, 3 and 4 cm. We also analyzed the data using a previously published method that divided the breast into 2 regions to determine whether the results differed to our more localized density method.
Results: Mean (SD) age of diagnosis was 46.8 (3.1). Results are presented for pre-diagnostic images taken 4.9 (3.3, 5.1) (median (inter-quartile range) years prior to diagnosis. We consistently found that as square-specific PD increased the odds of the square developing into a tumour increased. For example, using all possible controls in a breast and a grid length of 2 cm, the odds of a region becoming cancerous was 2.1 (95% CI: 1.1–3.8), 4.9 (2.8–8.6) and 6.4 (3.7–11.1) higher, respectively, for the second, third and top quartiles in PD within a woman's breast relative to the bottom one. Results were similar for images taken 3 years before diagnosis. The published method yielded a borderline

inconclusive association between region with greatest PD and subsequent tumour location (p = 0.07).
Conclusion: The findings help clarify the role of PD on breast cancer risk by suggesting that PD is a localized marked of risk.

37 Integration analysis between differentially expressed mRNA and miRNA induced by BRCA1 gene

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Background: Mutations within the BRCA1 tumour suppressor gene occur frequently in familial breast carcinomas and also decreased BRCA1 expression occurs in sporadic tumours. MicroRNAs (miRNA) are 20−25 nucleotide non-coding RNAs that inhibit the translation of targeted mRNA, and they have been implicated in the development of human malignancies, regulating a number of tumour suppressor genes (TSGs) and oncogenes. In our study we are to explore the relation between miRNA and the mechanisms of BRCA1 associated tumourigenesis.
Material and Methods: Whole genome transcriptional profiling covering >25,000 mRNA sequences and global miRNA expression profiling with >800 human miRNAs was performed on a Brca1 deficient, HCC1937 breast cancer cell line, and the isogenic HCC1937 expressing BRCA1. The miRNA targets were predicted with miRanda and TargetScan algorithms. Functional pathway enrichment was performed with the Ingenuity Pathway Analysis system.
Results: In our study we found over 8000 differentially expressed genes and 8 differentially expressed miRNAs between HCC1937 and HCC1937/BRCA1 cells (FDR $p < 0.05$). Subsequently, we integrated the mRNA and miRNA data to find statistically significant miRNA-mRNA relationships underlying the array signatures. Moreover, we identified a number of signaling pathways associated with these expression changes that included MAPK or NFκB pathway.
Conclusions: By this study we reveal the connection between miRNA, gene expression and pathways altered following expression of BRCA1 gene.

38 miR-449 induces apoptosis while triggering a stress and DNA damage response

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Background: The E2F1-responsive microRNA-34 family member miR-449 is a potent inducer of apoptosis[1], at least in part independently of p53. It displayed the highest expression in lung and trachea, while being strongly down-regulated in tumour cell lines, consistent with a tumour-suppressive activity. With this study, we aim to achieve a better understanding of the mechanisms leading to the induction of apoptosis, and to elucidate the role of miR-449 in normal cells.
Material and Methods: Using immunoblot analysis of H1299 cells (p53 −/−, non-small cell lung carcinoma) transfected with miR-449 or controls, we investigated the effects of miR-449 over-expression on potential target gene expression levels, on the DNA damage response, and on apoptosis-related pathways. The use of the caspase-inhibitor Z-VAD allowed us to discriminate between causes and consequences of caspase activation. In vitro cultivated aero-epithelial cells (AEC) were used to correlate the expression of miR-449 with the differentiation of bronchial epithelial cells.
Results: On top of inducing apoptosis and reducing CDK6 and SIRT1, miR-449 was able to accumulate gamma-H2AX, a common marker of DNA damage. The investigation of the DNA damage pathway revealed strong down-regulation of Chk1 and accumulation of phospho-p38-alpha. The siRNA knock-down of Chk1 alone was able to induce similar gamma-H2AX accumulation. Furthermore, strong up-regulation of miR-449 levels was observed upon differentiation of AEC.
Conclusions: Our results suggest that miR-449 leads to DNA damage accumulation through the down-regulation of an important cell cycle checkpoint, Chk1, thereby inducing apoptosis. Moreover, it may also target the Notch signaling linked protein DLL1, perhaps contributing to apoptosis or to bronchial epithelial differentiation, depending on the cellular context. E2F1-inducible microRNA 449a/b suppresses cell proliferation and promotes apoptosis.

Reference(s)

[1] Lizé M., Pilarski S., Dobbelstein M.; Cell Death Differ. 2010 Mar;17(3):452−8.

Sunday 27 June 2010 14:35−16:05

Presidential Session
Presidential Session II

39 Targeting the lactate transporter monocarboxylate transporter 1 constitutes a new therapeutic modality that disrupts a fundamental metabolic symbiosis in tumours

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Background: The glycolytic activity of hypoxic cells creates a gradient of lactate that mirrors the gradient of oxygen in tumours. In human tumours, high levels of lactate predict the likelihood of tumour recurrence, metastasis, and poor survival. In this study, we address the intrinsic contribution of the lactate anion to tumour growth and the tumour response to radiotherapy.
Materials and Methods: We initially selected SiHa and WiDr human cancer cell lines as metabolic archetypes of oxidative and glycolytic tumour cells, respectively. Metabolic profiling used enzymatic measurements and electron paramagnetic resonance oximetry. We used immunohistochemistry to detect the expression of the monocarboxylate transporter 1 (MCT1) in vitro and in vivo, including, under approval of the Duke University Institutional Review Board, in biopsies of human tumours. The significance of lactate uptake was tested by measuring intracellular pH, ATP level and cell survival, and by using the selective MCT1 inhibitor alpha-cyano-4-hydroxy-cinnamate (CHC) and specific siRNAs. The toxicity and therapeutic activity of CHC were tested in 3 different tumour models and mouse strains, with permission of local ethical boards. MCT1 inhibition and X-ray irradiation were used in combination to treat Lewis Lung carcinoma-bearing mice.
Results: We identified a metabolic symbiosis in tumours involving the recycling of lactate, released by glycolytic tumour cells, as an oxidative fuel for oxygenated tumour cells. The preferential use of lactate over glucose to fuel tumour cell respiration renders glucose available to fuel the glycolytic metabolism of hypoxic tumour cells. We further identified MCT1, selectively expressed at the plasma membrane of oxygenated tumour cells, as the prominent path for lactate uptake. We successfully disrupted the metabolic symbiosis by inhibiting MCT1 with a specific siRNA or with the selective inhibitor CHC, causing a switch from lactate-fueled respiration to glycolysis in oxygenated tumour cells. As a consequence, CHC delivery to tumour-bearing mice causes hypoxic/glycolytic tumour cell death by virtue of glucose starvation and the remaining oxygenated tumour cells were highly sensitive to radiotherapy. There was no overt toxicity. Validation of this new therapeutic strategy using and MCT1 expression in an array of primary human tumours provide clinical significance to anticancer MCT1 inhibition.
Conclusion: Tumours behave as metabolic symbionts that can be targeted therapeutically.
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40 TGFβ receptor inhibitors target the CD44high/Id1high glioma stem cell population in human glioblastoma

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Glioma is the most common tumour of the brain and its most aggressive form, called Glioblastoma multiforme (GBM), is one of the most aggressive and deadliest cancers with a median survival of around 14 months. In the last years a cell subpopulation have been described in GBM, the Glioma Stem Cells (GSCs), also called glioma-initiating cells. GSCs have characteristics similar to normal stem cells and are responsible for tumour initiation, relapse and therapeutic resistance. GSCs should then be considered critical therapeutic targets. In addition, it has been showed that TGFb signaling pathway has an important role in cancer. In particular, in high grade glioma, TGFb acts as an oncogenic factor.
Here, we show that TGFβ inhibitors, currently under clinical development, target the GSC compartment in human GBM patients. Using patient-derived specimens, we have determined the gene signature of TGFβ inhibition in human GBM which includes Id1 (inhibitor of differentiation 1) and Id3 transcription factors. Id1 has been shown to be expressed in B1 type adult neural stem cells where it has an important role in the regulation of the self-renewal capacity. More importantly, in cancer Id1 has been shown to be expressed in tumours and described to be involved in metastasis.
We have identified a cell population enriched for GSCs that is characterized by the expression of high levels of CD44 and Id1. The inhibition of the TGFβ pathway decreases the $CD44^{high}/Id1^{high}$ GSC population through the