48 Amplification and overexpression of vinculin are associated with increased tumour cell proliferation and progression in advanced prostate cancer

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Introduction: Patients with advanced prostate cancer are usually treated with androgen withdrawal. Although this therapy is effective at the beginning, nearly all prostate cancers become refractory to it. Approximately 15% of these castration-resistant (or hormone-refractory) prostate cancers harbor a genomic amplification at 10q22. Aim of this study was to explore the structure of the 10q22 amplicon and to determine the major driving genes.
Methods: We applied high-resolution array-CGH using the 244k Agilent microarrays to cell lines harboring 10q22 amplification. We identified the common amplified region (CAR) and silenced each of the genes in this region by an RNAi screen in the prostate cancer cell lines PC-3 and 22rv1. Genes with a significant growth reduction in the 10q22 amplified cell line PC-3 but not in the non-amplified 22rv1 cells were selected as putative candidate genes of this amplicon. They were further investigated in vivo by functional assays and in vitro by immunohistochemical analysis of the protein expression in more than 500 human prostate cancers on a tissue microarray (TMA).
Results: We were able to narrow down the CAR to a region of 5.8 Mb. The siRNA screening experiments revealed vinculin (*VCL*) as the most promising candidate gene of this amplicon. Immunohistochemical analysis of the vinculin protein expression on a TMA enriched for 10q22 amplified prostate cancers showed a strong association between *VCL* gene amplification and overexpression ($p < 0.001$). Further analysis of 443 specimens from across all stages of prostate cancer progression showed that vinculin expression was highest in castration-resistant prostate cancers, but negative or very low in benign prostatic hyperplasia ($p < 0.0001$). Notably, high tumour cell proliferation measured by Ki67 expression was significantly associated with high vinculin expression in prostate cancer ($p < 0.0001$).
Conclusions: Although there are countless reports on vinculin as a cytoskeletal protein, its protein expression or functional role in prostate cancer has previously not been investigated. Our data strongly suggest that vinculin is a major driving gene of the 10q22 amplification in prostate cancer and that vinculin overexpression might contribute to prostate cancer progression by enhancing tumour cell proliferation.

49 New functions for an old kinase: CK1 as a key player in p53, MDM2 and E2F-1 signalling pathways

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Background: The tumour suppressor p53 is a transcription factor that integrates distinct environmental signals including DNA damage, virus infection and metabolic stress into a common biological outcome that maintains normal cellular control. p53 is stabilised and activated by sets of enzymes that mediate covalent modifications. We previously identified a novel role for casein kinase 1 (CK1) as a p53-activating kinase in response to virus infection (MacLaine et al., J. Biol. Chem. 283, 28563−73). In this study we set out to (i) ascertain whether CK1 acts as a global p53 activator in response to a wide range of stresses and (ii) characterise the role of CK1 under normal, unstressed conditions.
Materials and Methods: MOLT-3 and A375 cells were treated with X-rays, Acadesine, inhibitors to CK1, ataxia telangiectasia mutated (ATM), AMP-activated protein kinase (AMPK) or MDM2 (D4476, KU-55933, Compound C or Nutlin-3, respectively), siRNA to CK1 or appropriate controls. Co-immunoprecipitation studies were performed in A375 cells.
Results: Inhibition of CK1 using the specific inhibitor D4476 did not attenuate the induction of p53 in response to either X-rays or altered ATP/AMP ratios, indicating that CK1 is a viral-specific kinase for p53. Instead, ATM and AMPK were identified as p53-activating kinases in response to ionising radiation and metabolic stress, respectively. However, depletion of CK1 using siRNA or inhibition of CK1 using D4476 activated p53 and destabilised E2F-1 under unstressed conditions, suggesting that steady-state levels of these proteins are controlled by CK1. Endogenous CK1 co-immunoprecipitated with p53, p53's negative regulator MDM2 and E2F-1, indicating the existence of a multi-protein complex. Treatment with the MDM2 inhibitor Nutlin-3 resulted in the same p53 and E2F-1 protein level changes as observed with D4476, highlighting a pharmacological similarity between MDM2 and CK1 small molecule inhibitors.
Conclusions: Distinct kinases mediate p53 phosphorylation in response to different stresses. CK1 represents the viral-specific p53-activating kinase with a previous independent study demonstrating that CK1 mediates the TGF-β activation of p53 (Cordenonsi et al., Science 315, 840–843). CK1 appears to function as a key switch, promoting MDM2-dependent p53 degradation and E2F-1 stabilisation under normal conditions, but disrupting p53-MDM2 complexes by phosphorylation in response to specific environmental insults. CK1 may therefore represent an attractive target for novel anti-cancer therapeutics aimed at reactivating the p53 pathway.

50 Cross-signaling of activated NF-kappaB and the tumour suppressor p53

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Background: NF-kappaB and p53 critically determine cancer development and progression. Defining the crosstalk between these transcription factors can expand our knowledge on molecular mechanisms of tumourigenesis.
Material and Methods: Cross-signaling of p53 and NF-kappaB was investigated using replicational stress- and TNFalpha-induced signaling by western blot, immunoprecipitations, viability assays, caspase assays, PI-FACS, immunohystochemistry, ABCD assays, EMSAs and chromatin immunoprecipitations. Several p53- or RelA-deficient cell lines were used as model system. Furthermore, cross-singnaling of mutated p53 with NF-kappaB was investigated in a novel murine pancreatic cancer cell model were mutated p53 is expressed from the endogenous promoter or deleted by homologous recombination.
Results: We show that induction of replicational stress activates NF-kappaB p65 and triggers its interaction with p53 in the nucleus. Experiments with p53- as well as p65-deficient cells revealed that both are required for enhanced NF-kappaB activity during S-phase checkpoint activation involving ATM and CHK1. Accordingly, the pro-inflammatory cytokine TNFalpha also triggers formation of a transcriptionally active complex containing nuclear p65 and p53 on NF-kappaB response elements. Gene expression analyses demonstrated that, independent of NF-kappaB activation in the cytosol, TNF-induced NF-kappaB-directed gene expression relies on p53. Remarkably, data from gain- and loss-of function approaches argue that survival function of NF-kappaB p65 is constitutively evoked by a p53 hot-spot mutant frequently found in tumours.
Conclusions: Our data suggest that p53 is unexpectedly necessary for NF-kappaB-mediated gene expression induced by atypical and classical stimuli. In addition, mutated p53 uses p65 to gain function in our model, suggesting an explanation for the question why p53 mutations rather than p53 deletions arise in tumours of various origins.

Sunday 27 June 2010 17:05−17:55

Award Lecture: EACR Cancer Researcher Award

51 Interplay between apoptosis and autophagy in the control of tumour cell death

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Inactivation of cell death pathways is a central component of cancer progression. Various mechanisms exist in normal human cells to invoke cell death and eradicate damaged cells that may otherwise multiply and form a tumour. The inactivation of these pathways during the genesis of cancer also poses problems for many forms of chemotherapy which utilize the same pathways to cause tumour cell death. The identification therefore of novel cell death regulators may lead to better diagnosis, better therapy choice and ultimately new targets for therapeutic intervention.
In order to identify novel cell death regulators we have undertaken a variety of forward and reverse genetic screens. As a result of these screens we identified a novel protein which we termed DRAM1 (for **D**amage-**R**egulated **A**utophagy **M**odulator1). DRAM1, which belongs to a previously undescribed family of human proteins, regulates cell death downstream of p53 and was the first target gene of p53 to be identified which modulates a process termed 'Autophagy'. Studies of cell death have classically focused on the evolutionarily conserved programmed cell death called apoptosis. More recently, however, it has become clear that cell death is also regulated by another evolutionarily conserved process – autophagy. Autophagy (or literally 'self-eating') like apoptosis is also a highly ordered process and operates at basal levels under normal conditions as a means of degrading long-lived proteins and damaged organelles. In contrast to apoptosis, however, there appear to be context specific aspects to autophagy, with reported involvement in both cell death and cell survival. Importantly too, autophagy is also involved in many other disease states beyond cancer, making the selective targeting of autophagy in cancer potentially difficult. To address this issue we have conducted an RNAi screen

in Drosophila for signaling pathways that regulate autophagy under hypoxic conditions – a state associated with many solid tumours. This identified a cell autonomous mechanism in human tumour cells which selectively promotes hypoxia associated autophagy and cell survival. Theses findings therefore represent a paradigm for the targeting of cancer-associated autophagy to cause tumour cell death.
The mechanism of action and context-specific nature of this and other factors identified from our screens will be described and the potential implications for the development and treatment of cancer will be discussed.

Sunday 27 June 2010 17:55–18:45

Radium Hospital Foundation Lecture: Genetic instability

52 Early epigenetic and genetic events in breast carcinogenesis

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The active acquisition of epigenetic changes is a poorly understood but important process in development, differentiation, and disease. Our work has recently demonstrated that repression of the p16/pRb pathway in human epithelial cells, a condition common to stem cells and many tumour cells, induces dynamic epigenetic remodeling resulting in the targeted methylation of selected CpG islands. We hypothesized that cells in this epigenetically-plastic state can be programmed by the microenvironment to acquire epigenetic changes that promote tumourigenesis. Normal human mammary epithelial cells (HMEC), and HMEC with repressed p16 were first transduced with constitutively active Ha-rasV12. In order to mimic the secretory aspects of the extracellular environment, the cells were subsequently cultured in a serum-rich environment. When p16-repressed cells were challenged with oncogenic stress, they failed to undergo the classic proliferative arrest as documented in normal cells. When further stressed by being cultured in a serum-rich environment, they spontaneously immortalized and exhibited phenotypic changes indicative of epithelial to mesenchymal transition (EMT). The EMT was accompanied by *de novo* methylation of the E-cadherin promoter and increased motility. These data demonstrate that signals from the microenvironment can induce phenotypic and gene expression changes that result in *de novo* epigenetic alterations important in tumour progression.

Sunday 27 June 2010 09:45–17:30

Poster Session

General, Molecular and Genetic Epidemiology

53 Assessing interaction between established breast cancer genetic susceptibility loci and selected non-genetic risk factors using data from the Breast Cancer Association Consortium

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Background: Breast cancer is known to have both a genetic and non-genetic etiology. A number of common genetic susceptibility variants has recently been identified by predominantly genome-wide approaches, but it is not known whether the loci involved act independently of established non-genetic risk factors. We aimed to assess interaction between genetic and non-genetic risk factors in the Breast Cancer Association Consortium. We focused on age at menarche, ever having had a live birth, number of live births, age at first birth and body mass index and their interaction with single SNPs within each of 10 established susceptibility loci (CASP8, FGFR2, 8q24, TOX3, MAP3K1, LSP1, 2q35, 5p12, SLC4L1 and COX11) and two additional SNPs (TGFB1 and ESR1) with less clear evidence of association.
Material and Methods: Per-allele odds ratios (OR) for SNPs were estimated by categories of non-genetic variables using logistic regression adjusted for study, and two-way gene-environment interaction was tested for by fitting a single-parameter interaction term for departure from log-additive effects. These analyses were applied to data from 14,600−29,991 cases and 16,188−30,990 controls from the Breast Cancer Association Consortium.
Results: No statistical evidence of interaction was observed beyond that expected by chance, given the number of tests carried out. The analyses were repeated using data from 11 population-based studies only, with similarly null results for interaction.
Conclusions: This is by far the largest study to assess interaction between established common genetic risk factors for breast cancer and age at menarche, parity-related variables and body mass index. Further studies of very even larger samples are required to determine whether these common susceptibility variants are associated with different risks of breast cancer depending on other non-genetic factors.

54 Screening for large genomic rearrangements of the BRIP1 and CHK1 genes in Finnish breast cancer families

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Background: In search for susceptibility genes that could explain a portion of familial breast cancer clustering in Finland, we set out to evaluate the presence of large genomic rearrangements in two candidate genes. BRIP1 (alias FANCJ or BACH1) is a BRCA1 associated protein mutated in a fraction of familial breast cancer and Fanconi anemia (FA) cases. The role of large *BRIP1* deletions in breast cancer predisposition is not well-characterized. CHK1 is a critical maintainer of cell cycle checkpoints and genomic stability, and is also involved in the BRCA1 and FA signaling pathways. Although CHK1 is an essential protein for cell cycle and DNA integrity maintenance control, no mutations in this gene has yet been associated with predisposition to cancer. To our knowledge, this is the first report to determine the existence of large *CHK1* deletions in familial breast cancer or in any disease with hereditary background.
Material and Methods: Blood DNA from affected index persons of 111 northern Finnish breast cancer families was assessed for possible constitutional exonic deletions or amplifications in the *BRIP1* and *CHK1* genes by using the multiplex ligation-dependent probe amplification (MLPA) method.
Results: Neither of the genes examined showed any large genomic rearrangements.
Conclusions: Our current results raise the possibility that germline exonic deletions or amplifications do not serve as a significant inactivating mechanism of the tumour suppressive functions of *BRIP1* and *CHK1* in breast tissue. Together with a PCR-based mutation analysis of the *BRIP1* gene, we conclude that pathogenic germline alterations in *BRIP1* appear not to contribute to breast cancer susceptibility in Finland.

55 Association of mammographic density with selected nutrients in Norwegian women

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Background: Mammographic density has been strongly associated with breast cancer risk. The determinants of mammographic density have been shown to be similar to those of breast cancer risk. Investigating the association between diet and mammography density could shed light on the possible relationship between diet and breast cancer risk.
Material and Methods: In this study we analyzed data of 2250 postmenopausal Norwegian women aged 50−69 years residing in the three largest Norwegian counties who participated in the Norwegian Breast Screening Programme in 2004. We estimated intake of selected nutrients and vitamins using a previously validated 200 item food frequency questionnaire. Mammographic density was assessed on scanned mammograms using a computer assisted method. We used multivariate linear regression to determine the least square mean of percent and absolute mammographic density adjusting for potential confounders. Because of the strong confounding effect of body mass index (BMI) on mammographic density we used different adjustments for BMI, analyses were also carried out for different BMI strata.
Results: The mean percent and absolute mammographic density were 19.2% and 24.5cm^2 respectively. Overall, we observed no strong association between mammographic density and total caloric intake or intake of proteins, carbohydrates, total fat, monounsaturated and polyunsaturated fat, cholesterol or dietary fiber. There was a positive borderline statistically significant association (p for trend=0.07) with saturated fat, which became stronger when the analysis was restricted to women with a normal BMI (23–25.9) (p for trend=0.01).