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

C. Ruiz[1], D. Holz[2], M. Oeggerli[1], S. Schneider[1], J. Kiefer[2], I. Gonzales[2], T. Zellweger[3], M. Barrett[2], D. Azorsa[2], L. Bubendorf[1]. [1]*University Hospital Basel, Institute for Pathology, Basel, Switzerland,* [2]*Translational Genomics Research Institute, Pharmaceutical Genomics Division, Scottsdale, USA,* [3]*St. Claraspital, Division of Urology, Basel, Switzerland*

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

N.J. MacLaine[1], A.S. Huart[1], D.W. Meek[2], T.R. Hupp[1]. [1]*CRUK p53 Signal Transduction Group, Edinburgh Cancer Research Centre, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom,* [2]*Biomedical Research Centre, Ninewells Hospital and Medical School, University of Dundee, Dundee, United Kingdom*

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

G. Schneider[1], A. Henrich[1], V. Wolf[2], M. Wieczorek[2], S. Reichardt[2], R.M. Schmid[1], F. Weih[3], D. Saur[1], T. Heinzel[2], O.H. Krämer[2]. [1]*Klinikum rechts der Isar, II. Medizinische Klinik, München, Germany*, [2]*Friedrich-Schiller-University Jena, Institute of Biochemistry and Biophysics, Jena, Germany,* [3]*Leibniz-Institute for Age Research, Fritz-Lipmann-Institute, Jena, Germany*

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.

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Award Lecture: EACR Cancer Researcher Award

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

K.M. Ryan[1]. [1]*The Beatson Institute for Cancer Research, Tumour Cell Death Laboratory, Glasgow, United Kingdom*

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