859 Constitutive DNA-damage signaling promotes cancer cell proliferation through Chk1-CIP2A pathway

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Background: DNA damage is a hallmark of malignantly transformed cells and over expression of the DNA damage sensitive kinases (transducers) has been observed in different human cancers. However, it is unclear whether constitutive DNA damage present in unperturbed cancer cells promotes tumourigenesis. Cancerous Inhibitor of Protein Phosphatase 2A (CIP2A) is a recently identified human oncoprotein that promotes malignant cell growth and cellular transformation (Junttila et al, Cell, 130, 2007).
Methods: The effects of Chk1 on CIP2A's expression, and on cell proliferation, were investigated in several cancer (gastric, breast, prostate and cervical) cell lines using Chk1 specific small interfering RNAs and chemical inhibitors for Chk1. CIP2A expression at the protein and mRNA level were studied by western blot and qRT-PCR analyses, respectively. MTT assay was used for evaluating the cell viability of the cancer cell lines. Tissue microarrays consisting of human ovarian cancer and human gastric adenocarcinoma specimens were evaluated for the presence of both Chk1 and CIP2A proteins using immunohistochemistry. Meta-analysis for Chk1 and CIP2A oncoproteins was done using Oncomine database.
Results: Here we show that either chemical or genetic inhibition of DNA damage sensitive kinase Chk1 results in potent inhibition of CIP2A protein expression levels in various cancer cell lines. Moreover, Chk1 activity was shown to promote proliferation of several cancer cell types. Importantly, Chk1 siRNA-elicited inhibition of cell proliferation is rescued by over expression of CIP2A from heterologous promoter. Moreover, in clinical tumour samples there is significant correlation between CIP2A and Chk1 expression. Furthermore, like CIP2A, increased Chk1 expression is shown to correlate with tumour progression in human cancers. Intriguingly, meta-analysis of seventeen published genome wide studies on different types of cancers unveils a striking similarity in the gene expression (mRNA from patients) pattern of both CIP2A and Chk1. Amongst these 12/17 studies show over expression (top 10%) of both these proteins.
Conclusions: Taken together these results identify a novel function for DNA damage kinase Chk1 in stimulating expression of human oncoprotein CIP2A. In general, these results suggest towards an unprecented molecular mechanism by which constitutive DNA damage present in cancer cells may promote tumourigenesis. Importantly, these results further support the role for both Chk1 and CIP2A in tumourigenesis and as target proteins for future cancer therapies.

860 Spi-1/PU.1 accelerates replication fork elongation and favors accumulation of genetic mutations in a multistep myeloid leukemia model

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Carcinogenesis involves the acquisition of multiple genetic changes altering various cellular phenotypes. Mechanisms that potentiate the formation of mutations are critical to accumulate all the mutations necessary to become a cancer cell and progress into a clinical cancer within human lifespan. Alteration of DNA replication, mainly at the level of initiation of replication, is one of these mechanisms, that has been directly involved in oncogenesis. Here, we have used a multi-step model of leukemia to determine the relationship between cancer progression and replication control. This model associates, at least, two molecular events; mutation in the *spi-1* gene causing a differentiation arrest and a second mutation in the *c-kit* gene conferring self-renewal properties on the cells. We have shown that Spi-1 increases the speed of replication by acting specifically on elongation rather than affecting origin firing. As a consequence, S phase duration was reduced in precancerous cells overexpressing Spi-1. Spi-1 acts by attenuating the program of replication control through Chk1 repression, independently of the ATR signaling pathway. Instead, PP1 phosphatase participates to this effect. Spi-1 promotes expression of the PP1ca phosphatase. Inhibition of PP1 activity triggers Chk1 activation and reduces the speed in the S phase progression, while overexpressing PP1 inhibits Spi-1 effect on the replication progression. These findings strongly support that PP1 is a link between Spi-1 and Chk1 revealing a new way for an oncogene to deregulate the DNA replication. Moreover, the diminution of Chk1 activity and the acceleration of replication did not result in DNA strand breaks but is associated with accumulation of genomic mutations. Our results suggest an new mechanism by which Spi-1 may promote oncogenesis, by controlling DNA replication and causing genomic mutability. We hypothesized that *Spi1* oncogene may act dually by accelerating the acquisition of oncogenic mutations and by displaying oncogenic functions; such as promoting survival and blocking differentiation in the initial steps of the tumourigenesis.

861 Escherichia coli isolated from Crohn's disease patient induces a HIF-1 dependant angiogenic and inflammatory response

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Background: Many cancers occur after chronic inflammation. This is particularly true in the gastrointestinal system in which inflammation could be induced by microbial pathogens.
Materials and Methods: Experiments were performed in human intestinal epithelial cell lines infected with the Crohn disease-associated *Escherichia coli* LF82 (AIEC) strain. Production of cytokines and pro-angiogenic factors in cells infected with LF82 bacteria was assayed by pPCR and ELISA. Isogenic mutant strains were used to characterize bacterial virulent factors involved in pro-inflammatory and pro-angiogenic responses.
Results: Here, we demonstrated that exposure of T84 cryptic-like intestinal epithelial cells to Crohn disease-associated LF82 bacteria induced the expression of HIF-1a, a central mediator of cellular adaptation to hypoxia and key factor in cancer development. Using RNA interference we showed that bacteria-induced HIF-1a regulated the expression of VEGF and IL-8, thereby pointing to a role for HIF-1 in angiogenesis and inflammation. Further, using isogenic mutant strains, deleted for the expression of type 1 pili and flagella, we identified flagella as the bacterial structure involved in cellular responses. Indeed, a blocking antibody against TLR5 receptor, recognized by flagella, inhibited LF82-induced VEGF and IL-8 production. Further, we showed that PI3-K and NF-kB-dependant signalling pathways accounted for VEGF and IL-8 expression. Finally, results obtained *in vivo*, using CEABAC10 transgenic mice that over-expressed CEACAM-6 protein involved in LF82 adherence, will be discussed.
Conclusion: We propose that LF82-induced HIF1a expression could promote the pro-angiogenic and pro-inflammatory responses that are observed in patients with inflammatory bowel disease and that these cellular responses could promote onset of colon cancer.

862 Association of polymorphisms in BRCA1 gene with breast cancer risk in Bulgarian familial cases

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Background: Studies in different populations worldwide demonstrated that germ-line mutations in *BRCA1* and *BRCA2* account for the majority of hereditary breast and ovarian cancer. In addition polymorphic variants in the main cancer susceptible genes have been implicated in increased breast cancer risk.
Materials and Methods: We have performed a case-control study of 8 polymorphisms in *BRCA1* gene, previously associated with breast cancer, (Q356R, D693N, S694S, L871P, E1038G, K1183R, S1613G and M1652I). The studied group consisted of 80 breast cancer patients with family history of breast cancer or early onset of the disease and 80 healthy control women, matched by age and ethnicity to the patients. The genotyping was performed by direct sequencing and the results analysed by chi-square test for association and odds ratio using Vassar Stats Calculator.
Results and Discussion: Two of the studied polymorphisms in the *BRCA1* gene demonstrated association with breast cancer risk. The genotypes CC of L871P (OR = 2.235; P = 0.01) and AA of E1038G (OR = 1.8; P = 0.02) appeared to increase twice the risk of breast cancer. The presence of one C allele in L871P (OR = 1.6; P = 0.05) was also associated with increased risk, whereas the genotype C/T (OR = 0.475; P = 0.02) and the presence of one T allele (OR = 0.626; P = 0.05) in L871P appeared to have protective effect. The genotypes CC of S694S (OR = 1.6; P = 0.08), GG of K1183R (2.182; P = 0.161) and G/A (OR = 2.3; P = 0.19) demonstrated tendency to increase the risk of breast cancer although the results did not show statistical significance.
Conclusions: Our results suggest significant association of L871P and E1038G SNPs in the *BRCA1* gene with increased breast cancer risk. An extended case-control study is necessary to confirm the contribution of the studied polymorphic variants to the risk of breast cancer development in the Bulgarian population.