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CIP2A (Cancerous Inhibitor of Protein Phosphatase 2A) promotes gastric carcinogenesis

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Cancerous Inhibitor of Protein Phosphatase 2A (CIP2A) is a recently identified human oncoprotein that promotes malignant cell growth and cellular transformation (Junttila et al, 130, Cell 2007). However, the clinical role of CIP2A in human malignancies has not been studied as yet.

Results of this study provide first evidence that CIP2A is a prognostic factor in human malignancies. We show that CIP2A immunopositivity associates with poor prognosis in certain subgroups of gastric cancer patients. Depletion of CIP2A in gastric carcinoma cells (AGS, MKN-28, and KATO-III) inhibits their proliferation. CIP2A also promotes c-Myc stability in gastric cancer cells, and these cells are dependent on c-Myc for their proliferation. Importantly, depletion of c-Myc inhibits CIP2A expression levels (protein and mRNA) and c-Myc activation results in increased CIP2A mRNA expression levels.

Taken together these results reveal for the first time the clinical role of CIP2A in a human malignancy. Moreover our results describe a novel positive feedback mechanism between CIP2A and c-Myc.

Altogether; our results indicate that inhibition of CIP2A could be a viable therapeutic approach in gastric cancer patients with CIP2A positive tumours.

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FGF9 mutations in colorectal and endometrial cancers

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Fibroblast growth factor 9 (FGF9), initially identified as a glia activating factor, has diverse effects in development and carcinogenesis. It maps to 13q11-12 which is a common area of loss of heterozygosity (LOH) in colorectal carcinomas. Here, we report 10 FGF9 mutations out of 203 colorectal and endometrial tumors and cell lines. One of these mutations (c.563delT) was detected in five different carcinomas (four colorectal and one endometrial). It causes a frameshift and creates a premature stop codon that deletes the last 4 amino acids ($FGF9^{\Delta 205\text{-}208}$). The other mutations included four missense and one nonsense. Analysis of the crystal structure of the mutant proteins predicted that all mutations should lead to loss-of-function. Further analysis of the biological activity of three of these mutations (p.V192M, p.D203G and $FGF9^{\Delta 205\text{-}208}$) showed that the mutant proteins have impaired ability to activate MAPKs cascade in cultured cells expressing FGF receptors. Consistent with the predicted loss-of-function, we observed LOH in 7/9 FGF9 mutant tumors. To our knowledge, this is the first report of somatic FGF9 mutations in human cancers. Further studies will clarify the extent and significance of these mutations in carcinogenesis.

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Mouse mammary tumour virus like-virus (MMTV-LV) is present in human prostate, ovarian and endometrial cancers but not lung cancer

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Background Mouse mammary tumor virus (MMTV) is a hormonally regulated, oncogenic virus, responsible for 95% of breast cancer in mice. Several groups have detected human MMTV-like virus (MMTV-LV) envelope (env) gene sequences with 95% homology to MMTV in approximately 40% of human breast cancers. We hypothesized that local hormonal effects might be of primary importance in determining the presence of MMTV-LV in human cancers and have investigated the prevalence of MMTV-LV in human prostate, ovarian, endometrial and lung cancers.

Materials and Methods The prevalence of MMTV-LV env DNA was detemined using nested PCR in 147 prostate, 75 ovarian, 30 endometrial and 30 lung cancers. The MMTV-LV long terminal repeat (LTR) region sequence from 14 prostate cancers and 14 ovarian cancers was compared to 6 published MMTV-like viral sequences from human breast cancer to study sequence variation.

Results MMTV-LV env DNA was detected in prostate cancers (59/147), ovarian cancers (15/75), and endometrial cancers (4/30) but not in lung cancers (0/30), suggesting a dependence of MMTV-LV on hormonally influenced tissues. There was no statistical difference in the rate of MMTV-LV env prevalence previously observed in breast cancers (107/346) to that observed in prostate, ovarian and endometrial cancers. Phylogenetic analysis of the MMTV-LV LTR sequence showed no clustering of the isolates according to tissue type, suggesting that MMTV-LV isolated from different tissues was the same virus with a varied tropism.

Conclusions Unlike the mouse model, MMTV-LV env sequences were detected in human cancers other than breast cancer. This indicates MMTV-LV expression is not breast cancer-specific and may relate to hormone-influenced viral expression, rather than an aetiological role. The co-localization of MMTV-LV with hormone receptors will provide further support for this association.

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hCCR4/CNOT complex targets DNA damage signalling pathway after genotoxic stress

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Cancer is one of the leading causes of mortality in developed countries. Platinum-based drugs are among the most active anti-cancer agents, and have been widely used in the treatment of a variety of human tumours. Apart from toxicity, resistance to chemotherapy limits the effectiveness of cancer treatments. Some patients do not respond to chemotherapy treatments and others relapse after a short period of response. Cisplatin, as with other anticancer agents, induces the DNA damage response and also activates a stress signalling pathway. An improved knowledge of the mechanisms underlying the resistance to treatment would generate new therapeutic strategies. The DNA-damage response network is highly complex and involves a multitude of proteins that sense the damage, transduce signals into cells and execute cellular responses. We have done a functional approach, based on Genetic suppressor elements (GSEs) strategy, in order to find new genes involved in cisplatin sensitivity. GSEs are short, biologically active, cDNA fragments that interfere with the function of their cognate gene. Using cisplatin as a selection marker, we identified the hCCR4 /CNOT6 gene that mediates cellular sensitivity to the drug. The precise role of the Ccr4-Not complex has not been determined, but seems to serve as a platform that regulates several different cellular functions in response to changes in environmental signals. Recently, CCR4 was described as playing a role in resistance to ionizing radiation and DNA damage, or replication stress induced by chemicals, in yeast Expression of hCCR4/GSE reduces hCCR4 protein levels in cells. However, mRNA levels are not affected, and this indicates that it could be affecting the protein function. The absence of CCR4 impacts on the sensitivity of mammalian and yeast cells to DNA-damaging agents. Our data indicate that hCCR4 plays a role in the control of cell cycle checkpoint following genotoxic stress; overexpression of hCCR4 appears to target Chk2 phosphorylation. Consequently, cells enter mitosis despite bearing DNA-damaged lesions; a higher proportion of phosphorylated H2A-X is detected in cells expressing hCCR4 when compared to WT controls. This finding introduces a new pharmacological target in the treatment of solid tumours.Our results point to a new protein involved in response-to-therapy and, as such, a new pharmacological target for chemotherapy.

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The EBV-encoded EBNA1 up-regulates macrophage migration inhibitory factor (MIF) and induces ERK signalling and the activation of Elk1 in B cell lymphoma

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Backround: The Epstein-Barr virus (EBV) is implicated in the pathogenesis of several germinal centre derived B cell malignancies including Hodgkin's lymphoma (HL) and Burkitt's lymphoma (BL). EBNA1 is an EBV protein that

is expressed in all virus-associated cancers; although it is required for efficient episome replication and segregation, it has also been suggested that EBNA1may contribute more directly to oncogenesis. To elucidate a possible role for EBNA1 in EBV-associated lymphomas, we performed a microarray analysis following the expression of EBNA1 in primary tonsillar germinal centre (GC) B cells, the presumed progenitor cell of HL and BL.

Materials and methods: HL and BL cell lines, primary tonsillar germinal centre (GC) B cells, AMAXA nucleofection, microarray, qPCR, western blot, ELISA, ELK1 activity by TransAM assay

Result: Microarray analysis revealed the up-regulation of macrophage migration inhibitory factor (MIF), an inflammatory cytokine that is increased in many cancers. MIF has been suggested to contribute to cancer progression through various mechanisms, including the activation of ERK MAP kinases. MIF is an attractive target for cancer therapies; several small molecule antagonists and monoclonal antibodies against MIF have already been developed.

We confirmed the up-regulation of MIF in EBNA1-expressing GC B cells by qPCR, and showed that MIF protein was also up-regulated in lymphoma cell lines after transfection of EBNA1 and in GC B cells after EBV infection. Furthermore, the up-regulation of MIF in these cells was accompanied by increased ERK and Elk1 activation. Further analysis of genes differentially expressed by EBNA1 in GC B cells revealed an enrichment of genes containing potential binding sites for Elk1. This suggests that the induction of MIF is responsible for many of the transcriptional changes induced by EBNA1. We are currently investigating whether EBNA1 induces ERK signalling pathway through MIF, and the extent to which Elk-1 activity is responsible for the subsequent regulation of various EBNA1 target genes.

Conclusion: Our data identify the oncogenic ERK MAP kinase pathway as a major target of EBNA1, and suggest that this virus protein can have important tumour-promoting effects aside from its role in viral episome maintenance.

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Proteomic analysis of beta-catenin activation in mouse liver identifies glucose metabolism as a new target of the Wnt pathway

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There is now accumulating evidence demonstrating a key role for Wnt/beta-catenin signalling in liver physiology and development. Aberrant activation of this pathway is one of the major genetic events observed during the process of hepatocarcinogenesis.

To gain insight into the mechanism underlying beta-catenin action in the liver, we conducted a proteomic study of the hepatic parenchyma of mice with inducible activation of the Wnt/beta-catenin pathway. We performed a quantitative differential proteomic study using 2D-differential in gel electrophoresis (2D-DIGE), combined with mass spectrometry of the liver proteome of hepatocyte-specific Adenomatous Polyposis Coli (Apc) knockout mice (Apc KOliv) compared to control mice.

We identified 94 protein spots showing differential expression (at least 1.5-fold difference, Student's t-test $p \leq 0.05$) between mutant Apc KOliv and control mice, corresponding to 55 different proteins. Most of these proteins were involved in metabolic pathways, highlighting the critical role of the Wnt pathway in the metabolic function of the liver. We previously demonstrated the key role of beta-catenin on ammonia metabolism, this study showed that the Wnt pathway also differentially regulates glycolysis and gluconeogenesis, revealing its role in glucose metabolism. We also identified protein targets of this pathway that are involved in the ER stress response, control of the cytoskeleton network and hepatocellular differentiation.

Thus, this proteome study allowed the identification of intermediary metabolic pathways that may be relevant in progression of liver tumors with aberrant activation of the Wnt pathway. Two metabolic systems targeted include glutamine metabolism and glucose metabolism, with an up-regulation of glycolysis. These two events result in an increase in the availability of glutamine, an important amino acid for the proliferation of tumor cells. A potential shift in energy metabolism towards glycolysis may confer a powerful growth advantage for hepatocellular proliferation during tumor progression.

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Overexpression and activation of hypoxia-inducible factor 1 through PI3K/Akt signaling pathway in human T-cell leukaemia virus type 1-infected T cells

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Background: HTLV-1 (human T-cell leukaemia virus type 1) is the causative agent of ATL (adult T-cell leukaemia). HTLV-1 Tax can activate the PI3K (phosphoinositide 3-kinase)/Akt signaling pathway, which is responsible for survival of HTLV-1-infected T cells. HIFs (hypoxia-inducible factors) are transcriptional regulators that play a central role in the response to hypoxia. Overexpression of HIF-1α in many cancers is associated with a poor response to treatment and increased patient mortality. Our objectives in this study were to investigate whether HIF-1 was activated in HTLV-1-infected T cells and to elucidate the molecular mechanisms of HIF-1 activation by focusing on the PI3K/Akt signaling pathway.

Materials and methods: We used HTLV-1-infected T-cell lines (MT-2, MT-4, SLB-1, and HUT-102) and uninfected T-cell lines (MOLT-4 and CCRF-CEM), and PBMCs (peripheral blood mononuclear cells) from healthy volunteers and patients with ATL to analyze expression of HIF-1α, HIF-1 DNA-binding, and transcriptional activity of HIF-1. The study protocol was approved by the Human Ethics Review Committee of the University of the Ryukyus, and all human samples were obtained after informed consent. siRNA (small interfering RNA) was used in HIF-1α knockdown experiments. Akt activity was analyzed by Western blot. LY294002 was used to inhibit PI3K/Akt signaling.

Results: Enhanced HIF-1α protein expression and HIF-1 DNA-binding activity were exhibited in HTLV-1-infected T-cell lines. Knockdown of HIF-1α by siRNA suppressed the growth and VEGF (vascular endothelial growth factor) expression in HUT-102 cells. HIF-1α protein accumulation and transcriptional activity of HIF-1 were enhanced by Tax. The HIF-1 transcriptional activity induced by Tax was inhibited by dominant-negative Akt. Importantly, mutant forms of Tax that are defective in activation of the PI3K/Akt pathway failed to induce HIF-1 transcriptional activity. The PI3K inhibitor LY294002 suppressed HIF-1α protein expression, HIF-1 DNA-binding, and HIF-1 transcriptional activity in HTLV-1-infected T-cell lines. In primary ATL cells, HIF-1α protein levels strongly correlated with levels of phosphorylated Akt.

Conclusions: The results of the present study revealed a potent pathway in which PI3K/Akt activation induced by Tax leads to activation of HIF-1 in the HTLV-1-infected T-cells under non-hypoxic conditions. As HIF-1 plays a major role in tumor progression, it may represent a molecular target for the development of novel ATL therapeutics.

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Metastasis-specific aptamers inhibit migration of cancer cells

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Cell migration plays a crucial role in metastasis. Therefore, the inhibition of cancer cell motility is a promising strategy for the treatment of cancer. It has turned difficult to block selectively the migration of cancer cells with pharmacological compounds. Here we demonstrate the possibility to use aptamers as anti-migratory agents. Aptamers are structured oligonucleotide ligands that bind their targets with high affinity and can inhibit their function. They are isolated by an in vitro selection referred to as Selex (Systematic Evolution of Ligands by EXponential enrichment) from large libraries of random oligonucleotide sequences.

We assumed that Selex, applied to aggressive cancer cells as a complex target, could generate functional aptamers against molecules participating in the achievement of migratory phenotype. We employed subtractive whole cell Selex to obtain aptamers against the molecular determinants of the metastatic phenotype of cancer cell. Two isogenic cell lines of Syrian hamster fibroblasts, transformed by the v-src oncogene (Deichman at al, 1989) were chosen. Both lines are highly tumorigenic in vivo, but differ by their capacity to produce lung metastasis (Deichman at al, 1989). High metastatic cell line HET-SR1 was targeted by Selex. In order to favour the isolation of metastasis-specific aptamers we applied a counter-selection step, where cell-specific and tumour-specific sequences were subtracted from the library after the binding with the low metastatic cell line HET-SR. 10 rounds of evolution under stepwise increasing selective pressure were performed. Binding efficiencies of resulting aptamers were determined and all binders were screened for their ability to inhibit migration of target cells in vitro.

It was shown that:

a) Selected aptamers specifically bind to the high metastatic HET-SR1 cells and are able to distinguish these cells from their low metastatic counterpart.

b) Several aptamers demonstrate capacity to inhibit cell migration in vitro. A few of them also inhibit cell invasion in vitro.

c) Interestingly, the active aptamer E10 binds to the cell surface, whereas another active aptamer, E37, is internalized into the cytoplasm. This suggests that functional aptamers exploit different ways for the achievement of their inhibitory effects.

Our data illustrates the potential of molecular evolution techniques in generating ligands targeting the metastatic phenotype of cancer cells.