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.

Rapid screening assays can help to define aptamers inhibiting desired cellular features. Further work scrutinizing the nature and mechanisms of the activity of functional aptamers in detail will contribute to increase the basic knowledge of the molecular determinants of metastasis and, in the future, may help to create new agents for prognostic or therapeutic purposes.

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Increased expression of VEGF in a prostate intraepithelial neoplasia (PIN)-like cell line leads to Epithelial-to-Mesenchymal transition (EMT)

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Prostate intraepithelial neoplasia (PIN) is thought to be the precursor lesion leading to invasive carcinoma. During this transition, several growth factors are overexpressed, including the vascular endothelial growth factor (VEGF), which plays a role in recruiting blood vessels. We have mimicked such a process in vitro using the PIN-like C3(1)/Tag-derived Pr-111 cell line. These cells are poorly angiogenic and exhibit very low tumorigenicity in vivo. Increased expression of VEGF164 in Pr-111 cells led to a significant increase in tumorigenicity as shown when inyected subcutaneously in nude mice, invasiveness when performed Boyden chamber assays, proliferation rates and angiogenesis. Moreover, VEGF164 induced strong changes in cell morphology and in the cell transcriptome, through an autocrine mechanism. Gene expression of 398 genes was significantly altered in VEGF164-overexpressing Pr-111 cells. These included genes related to modification of the actin filaments, cell adhesion, signal transduction, and proliferation. Pr-111-overexpressing cells also displayed features of epithelial-to-mesenchymal transition (EMT), with an increased expression of mesenchymal markers, such as N-cadherin and Vimentin. Levels of E-cadherin and B-catenin were not changed in these cells. Our results strongly suggest that increased expression of VEGF in malignant cells during the transition from PIN to invasive carcinoma leads to EMT through an autocrine loop, thus promoting tumor cell invasion and motility. Blockade of VEGF in PIN lesions would likely impair not only tumor angiogenesis, but also spread of malignant cells.

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The promoting effect of dietary lipids on experimental mammary cancer is accompanied by changes in the expression of differentiation-related genes

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Breast cancer is a significant cause of mortality in women worldwide. Environmental factors, specially diet and lifestyle, have an important role in its etiology. In this study we have investigated the effects of a high corn oil and a high virgin olive oil diets on tumour clinical, anatomopathological and molecular parameters related to differentiation. Female Sprague-Dawley rats were induced with DMBA and fed a low fat (LF, 3% corn oil), a high corn oil (HF-C, 20% corn oil) or a high olive oil (HF-O, 3% corn oil plus 17% olive oil) diet. The results showed that the HF-C diet reduced the latency time and increased the mammary tumour incidence, content and volume. On the other hand, the HF-O diet led to the opposite effects (higher latency time and lower tumour incidence, content and volume). Moreover, the HF-C diet increased the tumour morphological aggressiveness, whereas the HF-O diet led to adenocarcinomas displaying features of low morphological malignancy. These results showed a clear stimulating effect of the high corn oil diet on breast cancer and a negative modulator role for high virgin olive oil diet. We also analysed the effect of these diets on the expression of differentiation related genes. We studied the classical breast differentiation markers alpha- and beta-casein, finding that their expression was deregulated in the mammary tumours. We also investigated the protooncogene PCPH, observing that its expression pattern was associated to the cell differentiation degree of the mammary gland. Furthermore, this protein was down-modulated by effect of the HF-C. Finally, using cDNA microarrays we identified 3 genes down-modulated by the HF-C diet, but not by the HF-O diet: submaxilar alpha-2u globulin, VDUP1 and H19. These genes have also been related to differentiation. Our results suggest that changes in the modulation of genes with a role in differentiation can be one of the mechanisms by which the high corn oil and the high olive oil exert their differential modulatory effects on experimental breast cancer.

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Relationship between the methylation status of deleted in liver cancer-1 gene and invasiveness and metastasis of hepatocellular carcinoma

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Objective: To study the relationship between the expression level of the methylation Status of Deleted in Liver Cancer(DLC-1(gene and invasiveness and metastasis of hepatocellular carcinoma(HCC). Methods:76 surgical specimens and blood samples of patients with HCC were divided into high invasive and low invasive groups according to their clinical and pathological features. A methylation-specific PCR(MSP) was performed for the detection of hypermethylation of DLC-1 gene. Results: the expression level of the methylation Status of DLC-1 gene in surgical specimens of patients of HCC with high invasive group (n=40) was significantly higher than those with low invasive group(n=36)(÷2=4.3567, P < 0. 05), So was it in blood samples (÷2=4.4652, P < 0. 05)); Methylation Status of DLC-1 gene in HCC surgical specimens correlated with the clinical stages of patients with HCC((÷2=10.8478, P < 0. 05); Methylation Status of DLC-1 gene in blood samples from HCC patients correlated with their clinical stages(P < 0. 05), and with serum AFP level; The Median Survival Time were significantly different between HCC patients with the expression of the Methylated DLC-1gene and that without the expression of the Methylated DLC-1gene by follow-up (P < 0. 05). Conclusion: The expression level of the methylation Status of DLC-1 gene may play an important role in invasiveness and metastasis of HCC. Detection of the abnormal methylation of DLC-1 gene from HCC patients might not only offer an effective method for the auxiliary earlier diagnosis of the invasiveness and metastasis, but also serve as an objective target for HCC therapies.

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LMP1 gene variants of Russian origin from patients with EBV-associated pathologies and healthy individuals

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Background. Molecular analysis of Epstein-Barr virus (EBV) LMP1gene of different clinical and geographical origin allowed discovering two variants of gene (designated as LMP1-Cao and LMP1-C1510) possessing significantly higher transforming potential in comparison with their prototype LMP1-B95.8. Proteins encoded by these genes showed structural variations, consisting of multiple single base mutations, a C-terminal 30-base pair (bp) deletion and deletion of 15-bp unique sequence with insertion of three 33-bp repeats in the repeat region. On the basis of primary nucleotide structure and geographic origin LMP1 samples were classified as specific sequence variants.

As far as information concerning the strain differences of LMP1 EBV persisting in Russia was absent the main goal of present investigation was to carry out sequence analysis of LMP1 samples amplified from biological materials of Russian patients with EBV-associated diseases of lymphoid and epithelial origin, as well as healthy individuals.

Materials and methods. The DNA sequencing of tested LMP1 samples was carried out by means of reagents ABI PRISM® BigDye™ Terminator v. 3.1 with the subsequent analysis of the reaction products on automatic sequenator SEQDNA ABI PRISM 3100-Avant. The data processing was carried out with help of Chromas 230 and Vector NTI programs.

Results obtained allowed us to come to following conclusions: among point mutations detected in tested LMP1 samples the mutations I85L, F106Y, E328Q and S366T are predominant and possibly playing key role in modification of numerous signaling pathways as well as having important influence on transforming potential of the gene; individual variant of LMP1 for specific disease under investigations has not been detected; Cao-like deletions in LMP1 samples of Russian origin were detected occasionally in different studied groups; different genetic variants of LMP1 detected in different biological materials of the same patients possibly indicate both the of simultaneous persistence of genetically different variants of LMP1 as well as genetic drift of the original variant of gene as a result of mutations occurred de novo.

Conclusion. LMP1 samples amplified from Russian patients with HL, NHLs, IM, NPC and GC, as well as blood donors represent genetically heterogeneous group consisting of both earlier described in literature and possibly new variants of the gene.

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