clinical results. Finally, we simulated putative treatment schedules for identifying a possible effective treatment for GBM.
Results: Our simulation results successfully reproduce the experimental results of Kruse *et al*, (2001) for BTs grade III. Our model suggests several alternative schedules and dosages that manage to destroy the tumor. For a patient who died from a recurrence of BT grade III tumor, our model predicts that a longer treatment course may have been required to prevent tumor resurgence. The model interprets the failure of immunotherapy in the case of BT grade IV and predicts that a more intensive treatment protocol could eradicate GBM. We suggest alternative treatment courses for the eradication of GBM.
Conclusions: CTL immunotherapy is an effective therapy for BT grade III and IV. It can be optimized to prevent tumor recurrence. We believe that the experimental failure of Kruse *et al.* (2001) to treat GBM patients originated from immunotherapy not sufficiently intensive to overcome such a highly aggressive tumor (for example, using the same CTL dose we suggest daily infusions instead of every 4–5 days).

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Candidate tumor suppressor gene DLEC1 on 3p21.3 is hypermethylated in hepatocellular carcinoma

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Background: DLEC1 (previously also known as DLC1) is located at 3p21.3, which is one of the most frequent LOH regions in human chromosomes. It encodes a 1755-amino acid polypeptide and is localized only in the cytoplasm, with no homology to any known protein or domain. DLEC1 has been shown to have tumor suppressor function in cancer cell lines by colony formation assay. However, no alteration of the gene has been detected to cause dysfunction of its product in any of the cancers examined. Nevertheless, a CpG island has been found in the region of its promoter and first exon. Therefore, we tested the hypothesis that methylation of DLEC1 might suppress its expression to inactivate this tumor suppressor gene in hepatocellular carcinoma (HCC).
Material and Methods: HCC cell lines Hep3B, HepG2, Chang Liver, PLC/PRF/5 and SK-Hep-1, and 57 pairs of HCC primary tumors and matched adjacent normal samples were used. DNA methylation was detected by MSP and expression level by RT-PCR. Transfection of cell lines was mediated by Lipofectamine 2000 and transfected cells were selected by G418.
Results: DLEC1 is methylated in HCC cell lines Hep3B, HepG2, Chang Liver, PLC/PRF/5 and SK-Hep-1. The treatment of these cell lines with 5-aza-2'-deoxycytidine restored its expression. Using real-time RT-PCR and HCC primary tissues, we found that the expression level of DLEC1 in tumor samples was significantly lower than that in matched adjacent normal samples (t test, $p < 0.05$). Similarly, expression of DLEC1 in methylated samples was also significantly lower than that in unmethylated samples (t test, $p < 0.05$). Moreover, hypermethylation of DLEC1 was detected in 40 of 59 (67.8 %) primary tumors, while only 6 in 57 (10.5 %) nonmalignant specimens ($p < 0.001$, chi-square). We examined the relationship between DLEC1 methylation status and clinicopathological features, including age, gender, alpha-fetoprotein (AFP) levels, tumor size, ALTSG stage, AJCC stage, differentiation status, cirrhosis, encapsulation, vascular and capsule invasion in 49 samples with their tumor stages identified. The DLEC1 methylation status was associated with AJCC stages of tumors ($p = 0.036$, chi-square). Colony formation assay of exogenous expression of DLEC1 in cell lines showed that DLEC1 significantly inhibited cancer cells growth.
Conclusions: Our data showed that *DLEC1* is hypermethylated in the majority of hepatocellular carcinoma and able to suppress the growth of liver cancer cell lines, supporting its role as a tumor suppressor.

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Effect of IFN-α on gene expression: cDNA microarray analysis in human epidermoid cancer cells

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Introduction: Interferon-alpha a cytokine commonly used in the human cancer therapy, our research group is deeply involved in the characterization of the effects of IFNalpha activity on tumor cells. In details, we have found that IFN induces into human epidermoid cancer cells KB apoptosis and upregulates the expression of the Epidermal Growth Factor Receptor (EGFR) [1,2].
Materials and Methods: RNA Extraction; Probe Synthesis; Hybridization on cDNA Arrays; Statistical Analysis; Northern blot analysis; Quantitative Real Time PCR, Western blotting.
Results: In order to better characterize the molecular pathways that are elicited or suppressed by the action of IFN. A human 1.7k microarray (Microarray centre U.H.N., Canada) array was used for this experiment, which allows the simultaneous analysis of more than 1.5 thousand genes. Analysis of the hybridization signals through the use of a dedicated software (SAM and processed and analysed with MIDAS), has identified 25 differentially expressed genes: 19 down-regulated and 6 up-regulated in KB cells treated with IFN: prenylcysteine lyase:PCL, tissue inhibitor of met-alloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory):TIMP3, proteasome (prosome, macropain) activator subunit 1 (PA28 alpha), guanine monophosphate synthetase:GMPS. The differential expression of these genes in the two cell lines is being confirmed by northern blot analysis and quantitative RT-PCR as like the proteins expression.
Conclusions: This study represents a useful basis to define an extended molecular database of potential relevance both in basic cell biology studies and in therapeutic options.

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Tumour cells from stage III melanoma patients are often resistant to growth inhibition by Oncostatin M

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Oncostatin M (OSM) is an Interleukin-6 (IL-6) type cytokine originally described by its capacity to inhibit melanoma proliferation in vitro. However, OSM responsiveness is often lost in advanced stages melanoma cells. Here, the mechanisms involved in resistance to growth inhibition by OSM and IL-6 were analyzed for the first time on a large panel of metastatic melanoma cell lines (35). For 28% of the cell lines, OSM resistance correlated with the epigenetic loss of the OSM receptor beta (OSMRb) subunit. Treatment of these cells with the histone deacetylase inhibitor Trichostatin A re-established histone acetylation in the OSMRb promoter, expression of OSMRb and growth inhibition by OSM. Other defects linked to OSM resistance were identified, for 31% of the cell lines, on specific signal transduction pathways such as STAT3 (Ser727 phosphorylation), PKCa/b/d and/or AKT, explaining their co-resistance to OSM and IL-6. The use of PKCa/b/d inhibitors indicated that these serine kinases, together with STAT3, have a crucial role in growth inhibition by OSM. In nude mice injected with sensitive melanoma cell lines, OSM notably reduced tumour growth. Moreover, the patients whose melanoma cells were sensitive to growth inhibition by OSM and/or IL-6, and who were treated with tumor-infiltrating lymphocytes (as a potent source for these cytokines; $n = 13$), have a mean relapse-free survival of 8 years. Those whose melanoma cells were resistant to these cytokines ($n = 6$), have a mean relapse-free of only 15 months. Altogether, our results suggest a role for OSM in the prevention of melanoma progression in vitro and in vivo, and that metastatic melanoma cells could escape this growth control by the loss of OSMRb or defects on specific signal transduction pathways. We are currently validating on larger cohorts of patients, the involvement of IL-6 type cytokines in the response to immunotherapy and looking for a specific inflammatory state that could induce this cytokine resistance.

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The frizzled 8-related antiproliferative factor from IC patients inhibits bladder and kidney carcinoma cell proliferation in vitro

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Background: Antiproliferative factor (APF) is a potent sialoglycopeptide inhibitor of epithelial cell proliferation made by bladder cells from patients