demonstrated in mice and humans. Oligodeoxynucleotides containing unmethyled CpG motifs (CpG ODN), bind to the Toll-Like Receptor 9 (TLR9) and are powerful immunostimulating agents. We investigated whether tumor antigens released after ECT could efficiently prime the immune system and induce a systemic antitumor response when associated with CpG ODN.
Materials and methods: in a first set of experiments we analysed by immunohistochemistry the nature of the cellular recruitment induced after ECT and the expression of TLR9 mRNA by quantitative RT-PCR in tumors. In a second set of experiments, we investigated the effectiveness of the association ECT-CpG-ODN in two subcutaneous mouse tumor models: a fibrosarcoma (LPB) and a melanoma (B16F10). We studied both local and systemic anti-tumoral effects of this association using a model in which two tumors were inoculated but only one was treated. The specific immune response was further studied in the subcutaneous B16OVA tumor model.
Results: ECT induced the recruitment of CD11c and Mac1 positive cells expressing TLR9 in LPB tumors up to 72 hours after ECT. Our results showed a strong local efficacy of the ECT-CpG-ODN as well as antitumor effects on the controlateral non treated tumors in the two models. In nude mice, no effect was observed on tumors, suggesting a mechanism mediated by T lymphocytes. Moreover, the combination of ECT and CpG-ODN induced a 3-fold increase of specific anti-OVA CD8 lymphocytes in the tumor-draining lymph node, compared to ECT alone.
Conclusion: the combination of ECT, allowing tumor destruction, together with a suitable immunostimulating adjuvant could be a new strategy to treat patients with subcutaneous tumor localizations.

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Biological, histological and clinical impact of preoperative IL-2 administration in radically operable gastric cancer patients

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Background: Surgery induced lymphocytopenia and this decrease in host defences, related to IL-2 endogenous embalance during postoperative period could promote the proliferation of possible micrometastases and the implantation of surgically disseminated tumour cells. Moreover Tumor infiltrating lymphocytes (TILs), activated by endogenous IL-2 release, is linked to prognosis in cancer patients The aim of this randomized study is to assess the biological (peripheral blood cells count, related to the grade of immunosuppression), histological (TILs) and clinical (overall and disease free survival) impact of preoperative low doses administration of IL-2 in patients with radically operable gastric cancer.
Materials and methods: This prospectic study enrolled 89 consecutive patients with histologically proven gastric adenocarcinoma who underwent radical surgery from October 1999 to December 2003 (M/F 49/40; mean age 67; range 42–82). Patient were randomized to be treated with surgery alone as controls (45 patients) or surgery plus preoperative treatment with recombinant human IL-2 (44 patients). We considered the total lymphocyte count and lymphocyte subset (CD4, CD4/CD8) during the preoperative period, before IL-2 administration, and on the 14^{th} and 50^{th} day, peritumoral stromal (fibrosis) reaction, neutrophils, lymphocytes and eosinophils infiltration in tumor histology, and morbidity disease free and overall survival were evaluated.
Results: Two groups were well-matched for type of surgery and extent of disease. All the patients underwent radical surgery plus D2 lymphadenectomy. At baseline, there were no significant differences in total lymphocyte and lymphocyte subsets between groups. The control group showed a significant decrease of total lymphocytes, CD4 cells, and CD4/CD8 ratio at the 14^{th} postoperative day relative to the baseline value. In the control group 65% of patients had a decreased of CD4 under 500 cells/mmc. Instead it has been observed in IL-2 group a significant increase over the control group values of total lymphocytes and CD4 cells (14^{th} total lymphocytes and CD4: IL-2 vs control $p < 0.05$). Moreover in this group only 15% patients had CD4 under 500 cells/mmc. This difference, in CD4 count, is significant even at the 50^{th} postoperative day ($p = 0.006$). IL-2 group showed lower postoperative complications (4/44 vs 13/45; $p < 0.05$), and higher lymphocyte/eosinophil infiltration into the tumor ($p < 0.0002$). Median follow up was 36 months (range 12–72) and median overall and disease-free survivals were longer, even if not significantly, in the IL-2 group than in the control arm ($p = 0.07$ and $p = 0.06$ respectively).
Conclusion: This randomized study would suggest that a preoperative immunotherapy with IL-2 is a well tolerated treatment able to prevent surgery induced lymphocytopenia. IL-2 seems to neutralise the immuno-suppression induced by operation and so to stimulate the host reaction against tumour tissue (lymphocytes/eosinophils infiltration). Furthermore IL-2 seems to have an impact on clinical course reducing morbidity of surgery and ameliorating overall and disease free survival

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Mechanisms of transcriptional upregulation of DR5 by chemotherapeutic drugs and sensitization to TRAIL-mediated apoptosis

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TRAIL, a member of the TNF family, has been shown to kill sensitive tumor cells with minimal toxicity to normal tissues and is a new candidate for immunotherapy in the treatment of drug-refractory tumor cells. However, many drug-resistant tumor cells are also resistant to TRAIL and such tumors require sensitization to reverse TRAIL resistance. We, and others, have reported that several sensitizing agents (ex. CDDP, ADR, chemical inhibitors, etc.) in combination with TRAIL result in significant synergistic apoptosis, however the mechanisms by which this sensitization is achieved still remain unclear. Based on the observed upregulation of Death Receptor 5 (DR5) expression, induced by the sensitizing agents (*Ng et al., Prostate, 53: 286, 2002; Huerta-Yepez et al., Oncogene, 23: 4993, 2004*), we hypothesized that many of those drugs may, directly or indirectly, interfere with a repressor factor of the DR5 transcription.
Examination of the DR5 promoter revealed the presence of one binding site for the transcription repressor Yin Yang 1 (YY1), suggesting that YY1 may negatively regulate DR5 transcription. This hypothesis was tested by examining a luciferase reporter system (pDR5 wild type) and plasmids in which the YY1-binding site was either deleted (pDR5/-605), and/or mutated (pDR5-YY1 mutant).
Using the PC-3 prostate (androgen independent) tumor cell line as a model system, we showed that PC-3 transfected with pDR5 wild type resulted in basal luciferase activity, whereas treatment with CDDP or ADR significantly augmented luciferase activity. PC-3 cells transfected with pDR5/-605 or pDR5-YY1 also resulted in significant potentiation of the basal luciferase activity. Inhibition of YY1 by siRNA revealed increased sensitization of tumor cells to TRAIL-mediated apoptosis. Reduced YY1 DNA binding properties and downregulation of the NF-kB promoter activity were also shown to be triggered by drug treatment.
These findings indicate that YY1 negatively regulates DR5 transcription inducing tumor cells' resistance to TRAIL. They also support the hypothesis that drugs-induced upregulation of DR5 expression is mediated via inhibition of the transcription repressor YY1. On a clinical aspect, the above findings suggest that tumor cells overexpressing YY1 will be resistant to TRAIL-mediated apoptosis. Therefore, inhibition of YY1 may be clinically useful in the therapeutic application of TRAIL in resistant tumor cells.

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P43/EMAP-II expression in colorectal cancer is associated with hypoxia, enhanced lymphocyte infiltration and apoptosis

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Aims: P43/Endothelial monocyte-activating polypeptide II (p43/EMAP II) is a proinflammatory cytosine and a chemoattractant for mononuclear phagocytes and polymorphonuclear leukocytes, found in culture supernatants of many tumour cell lines. We recently demonstrated that p43/EMAP-II induces apoptosis in mitogen-stimulated lymphocytes, and suggested that it may be a constituent of a novel immune evasion mechanism employed by tumour cells [1]. Furthermore p43/EMAP-II release is enhanced by hypoxia [2]. Our study has examined the association between p43/EMAP-II expression and hypoxia in colorectal cancer (CRC), and also the association between p43/EMAP-II and lymphocyte apoptosis.
Methods: Formalin-fixed, paraffin-embedded archival tissue samples from a well-characterised population of 72 patients diagnosed with colorectal tumours were used in immuno-histochemical studies. Antibodies against p43/EMAP-II, carbonic anhydrase (CA IX) as a surrogate marker of hypoxia, and CD3 to identify tumour-infiltrating lymphocytes (TIL) were used. Areas of p43/EMAP-II and CA IX staining were quantified using computer-aided image analysis. Antibodies against active Caspase-3 and PARP were used to identify apoptosis in TIL.
Results: P43/EMAP-II expression was correlated with CA IX expression in CRC. Patients with high p43/EMAP-II expression seemed to do better than those with low, and the reverse was true for CA IX. There was also a positive correlation between p43/EMAP-II and the lymphocyte counts in CRC ($p = 0.03$), as well as between CA IX and lymphocyte counts ($p = 0.02$). The presence of CD3+ cells was a good prognostic indicator in terms of overall survival. There was a significant association between