cells compared to its resistant derivative cells, under 5-FU or TRAIL conditions. In contrast, D59 cells were sensitized by both molecules exposure simultaneously, witch increased level of apoptosis in the two cells. These results indicated that the function of the death receptors was partially unaffected in the D59 cells. So, we next investigated if modulation of the different death receptors could be involved in 5-FU apoptosis pathway. Although 5-FU treatment did not modulate mRNA of the DRs and using flow cytometry analyzes, we showed that 5-FU induction seem to increase the pro-apoptotic DR5 expression level on HCT116 cell lines whereas only the DcR1 seem to be regulated on D59 cells. In addition, siRNA–mediated down regulation of DcR1, were found to sensitize resistant colon cancer cells to the chemotherapeutic agent. In contrast, siRNA targeted of DR5 on HCT116 cells, rendered resistant these cells to apoptosis induced by 5-FU. Our work demonstrates that change in surface expression of death receptors might be a key determinant in acquired resistance to 5-FU and use of DcR1 targeted therapy might be a good strategy to overcome 5-FU chemoresistance.

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Do not die, become senescent: a new type of cellular resistance induced by topoisomerase II inhibitors in tumor cells with functional p53

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Cellular senescence is one of the mechanisms which prevents the development of cancer by eliminating cells which acquired potentially deleterious DNA mutations. Recent studies show that treatment of tumor cells with anticancer agents leads to permanent growth arrest with phenotypic features of senescent cells. In this study, we characterized effects induced by three different DNA topoisomerase II inhibitors (m-AMSA, ICRF-187 and triazoloacridone C-1305) in human lung carcinoma A549 cells. At minimal effective concentrations, all studied drugs induced permanent growth arrest in A549 cells which was accompanied by morphological and biochemical features of senescent cells, such as flat cell morphology, expanded lysosomal compartment and increased activity of senescence-associated form of β-galactosidase. Flow cytometry analysis showed that the majority of drug-treated cells arrested in G2/M and a substantial fraction of cells entered polyploidy. Expression of p53 and p21 dramatically increased in drug-treated cells as revealed by Western blot analysis. At the same time, expression of mitotic regulators, cyclin B1 and cdk1, as well as topoisomerase IIα and PARP-1 decreased to undetectable levels at drug exposure times longer than 72h.
It is believed that tumor cells which become senescent after exposure to antitumor agents are not able to regain the proliferative potential. However, after prolonged post-incubation of A549 cells (1–2 weeks) treated with minimal effective doses of topoisomerase II inhibitors, a small fraction of cells re-started cell proliferation. Interestingly, both the fraction of cells which were able to proliferate after drug treatment and the time required for proliferation recovery was different for studied topoisomerase II inhibitors. We have also shown that topoisomerase II inhibitors induced senescent phenotype followed by proliferation recovery only in cells with functional p53. In tumor cells in which p53 gene was inactivated, exposure to topoisomerase II inhibitors led to mitotic catastrophe and cell death.
Together, we propose that induction of long-term growth arrest in tumor cells by topoisomerase II inhibitors corresponds to a new type of resistance mechanism which is characteristic for tumor cells with functional p53 pathway. Further characterization of its molecular mechanisms could be important given that induction of drug-induced premature senescence is proposed to represent an alternative approach to treat human cancers.

Monoclonal antibodies and targeted toxins/nuclides

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In vitro and in vivo inhibition of functional responses at insulin-like growth factor-1/insulin hybrid receptors by h7C10, a novel humanized anti-IGF-1R monoclonal antibody

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Background: a novel monoclonal antibody (Mab, 7C10) was raised against the human insulin-like growth factor-1 receptor (IGF-1R); both murine and humanized (h7C10) Mabs exhibited potent inhibition of tumor growth in animal models (Goetsch et al., 2005). Further evaluation of their inhibitory activity at hybrid receptors (Hybrid-Rs) composed of the hetero-tetrameric association between IGF-1R and insulin receptor (IR) was performed using both *in vitro* approaches and *in vivo* animal models. Importance of Hybrid-Rs has repeatedly been reported as playing a potential role in various diseases including cancer.
Materials and Methods: R- mouse fibroblasts were stably co-transfected to express either IGF-1R or IR alone or both IGF-1R and IR, thereby expressing Hybrid-Rs. Pharmacological and biochemical *in vitro* assays were set-up to evaluate Mab activities such as [125I]IGF-1 and [125I]insulin competition binding to immuno-captured cell lysates, western blot analyses using specific antibodies to detect IGF-1R, IR or Hybrid-Rs phosphorylation, down-regulation of IGF-1R and Hybrid-Rs expression. *In vivo* experiments were performed in a xenograft model of MDA-MB-231, a non-estrogen dependent tumor cell line expressing comparable levels of IGF-1R and IR randomly assembled in Hybrid-Rs (Pandini et al., 1999), to compare the anti-tumor activity of h7C10 (binding to IGF-1R+Hybrid-Rs) with IR3 and 47–9 Mabs recognizing selectively IGF-1R and IR+Hybrid-Rs respectively.
Results: potent and full inhibition of [125I]IGF-1 binding was observed for 7C10 and h7C10 at both IGF-1R and Hybrid-Rs with affinities in the nanomolar range. On the other hand, [125I]insulin could not be displaced by these Mabs from its cognate receptor. Potent and efficacious inhibition of both IGF-1 and IGF-2-mediated IGF-1R and Hybrid-Rs phosphorylation was demonstrated. The response was similar to the control Mab 47–9. No modulation of the insulin- or IGF-2-mediated IR phosphorylation status was observed. Ligand-independent down-regulation of both IGF-1R and Hybrid-Rs was obtained upon long-term (24 hours) association with 7C10 Mab or its humanized form. Significant inhibition of the *in vivo* growth of MDA-MB-231 cells was observed with h7C10. Comparison between the *in vivo* activity of h7C10 with the one of IR3 and 47–9 Mabs showed that h7C10 had a significantly higher activity than that observed for the two other antibodies in the MDA-MB-231 model.
Conclusion: the herewith data clearly demonstrate that 7C10 and h7C10 selectively and efficaciously bind to both IGF-1R and Hybrid-Rs without affecting IR. They inhibit as well the functional signaling of IGF-1R/IR Hybrid-Rs regardless the activating ligand as well as mediate their down-regulation. These potent inhibitory properties are likely to participate in their *in vivo* anti-tumoral activities in xenograft models expressing both IGF-1R and Hybrid-Rs and may be of potential interest for therapeutic applications for the humanized Mab.

References

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A phase I trial incorporating the pharmacodynamic (PD) study of circulating tumour cells (CTC) of CP-751,871 (C), a monoclonal antibody against the insulin-like growth factor 1 receptor (IGF-1R), in combination with docetaxel (D) in patients (p) with advanced cancer

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Background: C is the first specific, fully human, monoclonal antibody to target IGF-1R in clinical trials. It potently inhibits IGF-1R signaling, enhancing D antitumor activity. This trial investigated the safety, feasibility, dose limiting toxicity (DLT), PK and antitumor activity of D administered with C every 3 weeks. PD studies evaluated CTC counts pre- and post-treatment and IGF-1R expression in CTC.
Methods: The C doses tested were 0.1, 0.4, 0.8, 1.5, 3.0, 6.0 and 10 mg/kg in sequential cohorts of 3–6 p. D was fixed at 75 mg/m^2. P achieving disease control continued on C alone if experiencing D toxicity.
Results: 27 p (26 male) have received 173 courses of C with D. 11 p received 6 or more courses of the combination. A further 34 courses of C alone have been administered. No grade (Gd) 3/4 toxicities has been attributed to C to date with the observed toxicities being attributable to D. Gd 3/4 toxicities were neutropenia (22/27 p) and neutropenic fever in 3/27 p. Gd 3 diarrhea was reported in 4 p, but this was easily controlled with antidiarrheals. Transient mainly Gd 1/2 hyperglycaemia was noted largely on day 1, following steroid premedication (20 p), but no significant C related hyperglycemia has been observed without steroids except for 1 p with Gd 2 hyperglycemia on C alone. An MTD has not been reached. Serial echocardiograms demonstrated no cardiac toxicity. Of 21 castration resistant prostate cancer (CRPC) p treated, 7 have had a confirmed PR, with 1 further unconfirmed PR. Six p have disease stabilization for >6 months (median number of courses: 10; range: 7–16). 10 p have maintained PR or SD with C alone for 1–7 courses. IGF-1R expression