response to siXL1 both at the molecular and survival levels suggesting that it remains possible to increase the effect of siXL1 by optimizing the delivery conditions.

Otherwise, considering that the concomitant inhibition of Bcl-xL and Mcl-1 expression could increase the efficiency of our strategy, as suggested in the literature, the interest of such combination was evaluated in vitro. We showed that neither siXL1 nor siMCL1 alone induced cell death whereas the combination of these siRNA induced a massive apoptosis. This observation shows that Bcl-xL and Mcl 1 appeared able to cooperate to protect ovarian carcinoma cells against apoptosis, either in response to oncogenic stress (providing a clinical advantage) or in response to chemotherapy. We are now analyzing whether the association of siXL1/siMCL1 with cisplatin could avoid the long term recurrence. Such multitargeted therapy could be also of interest for the treatment of ovarian carcinoma, in combination with conventional chemotherapy. These results are currently under in vivo preclinical validation.

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MCL-1 is an important determinant of the apoptotic response to the BH3-mimetic molecule HA14-1 in cisplatin resistant ovarian carcinoma cells

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Chemoresistance and in vitro recurrence of ovarian carcinoma have been previously associated to the absence of down-regulation of Bcl-xL expression in response to cisplatin. Our team is therefore developing various strategies to impede Bcl-x_L activity and/or expression, among which the use of BH3-mimetic molecules. These compounds reproduce the l structure of the BH3 domain of the Bcl-2 family members; they are able to induce apoptosis by dissociation of bax-like pro-apoptotic multidomain proteins from their anti-apoptotic partners.

We evaluated the interest of one of them, HA14-1, on various ovarian carcinoma cells lines resistant or sensitive to cisplatin. Differences were observed in response to HA14-1 in these cell lines, one of them undergoing strong apoptotic cell death (IGROV1-R10) whereas the others presented only a partial response (IGROV1, SKOV3, A2780) or an absence of response (OAW42). However, the sensitivity to HA14-1 was unrelated to the level of sensitivity to cisplatin, and the expression of HA14-1 targets (Bcl-2 and Bcl-x_L) was not correlated to these different responses.

In contrast, the lost of MCL-1 seemed associated with cell death in response to HA14-1, whereas maintenance or increase of MCL-1 expression led to resistance to this agent.

We therefore attempted about the importance of MCL-1 in the response to HA14-1 in SKOV3 and OAW42 resistant cells, and decided to inhibit its expression using a siRNA targeting MCL-1. Our results showed that siMCL-1 did not induced apoptosis on its own in these cells, whereas its association with HA14-1 induced a massive cell death. This results suggests that MCL-1 could cooperate with others Bcl-2 family members (e.g. Bcl-x_L) to protect ovarian carcinoma cells against oncogenic stress-induced apoptosis.

We also demonstrated that in SKOV3 cells (both resistant to cisplatin and to HA14-1), cisplatin was able to decrease MCL-1 expression, and that the association of HA14-1 with cisplatin induced a massive cell death, whereas cisplatin or HA14-1 alone was only transiently cytostatic.

These results suggest that MCL-1 is essential for the response to various apoptotic stimuli (oncogenic stress or conventional chemotherapy), and present resistant ovarian carcinomas as pertinent targets for the use of BH3-mimetics. Our work also showed that a siRNA directed against MCL-1 could interestingly reinforce the action of such molecules for the treatment of ovarian cancers refractory to conventional chemotherapy.

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Incorporation of a targeting ligand into adenovirus capsid as a mean of delivery of anti-angiogenic factors into tumors

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Inhibition of new vessels formation constitutes an approach to prevent tumor growth because angiogenesis is a mandatory step for tumor development. This can be achieved by delivery of genes able to modulate endothelial cells growth. Adenovirus (Ad) are potent tools to deliver genes because they can be produced at high titers and display high transduction efficiencies in a wide range of cell types. However, different studies indicated that gene transfer by Ad vectors into endothelial cells was confronted with poor transduction efficiency due to the lack of expression of the primary Ad receptor.

In order to improve gene transfer in endothelial cells, we inserted a targeting peptide (CNGRC motif) into different Ad capsid proteins. This peptide was previously shown to bind to aminopeptidase N (APN), a receptor expressed by neovessels. Compared to Ad bearing a wild-type capsid (Adwt), Ad bearing this peptide either into fiber protein (AdFNGR) or hexon protein (AdHNGR) were shown to achieve a higher β-galactosidase (β-gal) expression, in different endothelial cell lines (EAhy.926, SLK, CPAE) but also in human primary vascular endothelial cells. AdHNGR was also found to transduce more efficiently different tumor cells (LLC, RD and WEHI). We confirmed a role of APN in this new entry pathway since APN-specific uncompetititive (curcumin) and competitive (PC-18) inhibitors were able to reduce the ability of NGR-bearing Ad to transduce LLC cells. AdHNGR were also able to transduce some APN-negative cells (MDA-MB-435, L929) that are poorly transduced by Adwt. Binding assays studies showed that this property could be related to their ability to interact with αvβ3 integrins through NGR motifs.

Direct administration of AdHNGR into carcinoma (LLC) pre-established in nude mice led to a level of gene transfer comparable to Adwt. However, when AdHNGR was pseutotyped by an Ad3 fiber (targeting CD46, a receptor not expressed in mice), as a first approach of detargeting, we observed a 4-fold higher β-gal expression in LLC tumors compared to tumors injected with Adwt pseudotyped with an Ad3 fiber.

Altogether, our results pointed out that AdHNGR is very potent to transduce endothelial cells and emphasized that hexon protein could constitute a better alternative to fiber protein for incorporation of targeting ligands. Experiments are currently conducted to assess the ability of AdHNGR to deliver more efficiently angiostatin K1-5 into tumors and to decrease tumor growth.

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RNA interference-based strategies directed against Bcl-xL and MCL1 for the treatment of malignant pleural mesothelioma

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Malignant pleural mesothelioma (MPM) is a highly aggressive tumour with poor prognosis and limited response to standard combined chemotherapy (i.e. pemetrexed plus cisplatin). Anti-apoptotic proteins with altered expression have been previously described to contribute to chemoresistance, and among them, Bcl-xL seems to play an important role in MPM. Different strategies have been developed to impede its activity (BH3-mimetics) or its expression (antisense oligonucleotides). Interestingly, since it based on the highly specific and efficient silencing of a target gene, RNA interference (RNAi) represents one of the most promising innovating approaches to be combined to conventional therapies. In our study, a Bcl-xL specific RNA interference approach (siXL1) was used to inhibit Bcl-xL expression in mesothelioma cell lines for evaluating both its antitumor effect and its potential to sensitize mesothelioma cells to standard chemotherapy. We showed that siXL1 induced a drastic inhibition of Bcl-xL expression both at the mRNA and protein levels in different MPM cell lines. We characterized the response of chemoresistant NCI H28 cells to siXL1, alone or associated to cisplatin. siXL1 alone caused death of a fraction of the population (about 20%), the majority of cells being only transiently arrested in the cell cycle for few days. Notably, the combination of siXL1 and cisplatin resulted in a supra-additive effect with nearly complete annihilation of the population, whereas neither cisplatin alone nor cisplatin associated to control siRNA induced cell death in these cells. Although the observed cell death presented some features of apoptosis, its nature remains to be fully determined. Moreover, it was recently demonstrated that the neutralization of both Bcl-xL and MCL1 suffices for efficient Bak-mediated apoptosis. We thus evaluated the interest of the siXL1/siMCL1 combination and showed that this association is sufficient to induce a significant cell death. The interest of such siRNAs association combined to standard chemotherapy for the prevention of long term recurrence is under investigation. Finally, preclinical studies will be performed in nude mice to precise the therapeutic potential of such approaches for the treatment of MPM. In summary, these findings highlight that siRNA strategy aimed at down-regulating both Bcl-xL and MCL1 may be used as novel and highly effective tool, with the potential for future targeted therapy of malignant pleural mesothelioma.