

Bcl-x_L and MCL-1 constitute pertinent targets in ovarian carcinoma and their concomitant inhibition is sufficient to induce apoptosis

Emilie Brotin¹, Matthieu Meryet-Figuière¹, Karin Simonin¹, Raphaël E. Duval¹, Marie Villedieu¹, Johanne Leroy-Dudal¹, Ester Saison-Behmoaras², Pascal Gauduchon¹, Christophe Denoyelle¹ and Laurent Poulain¹

¹Groupe Régional d'Etudes sur le Cancer (EA 1772, Université de Caen), Unité "Biologie et Thérapies Innovantes des Cancers Localement Agressifs", Centre de Lutte Contre le Cancer F. Baclesse, Caen, France

²Inserm U565, Acides nucléiques: dynamique, ciblage et fonctions biologiques, Muséum National d'Histoire Naturelle, 57 rue Cuvier, Paris, France

In ovarian carcinomas, recurrence and acquired chemoresistance are the first leading causes of therapeutic failure and are responsible for the poor overall survival rate. Cisplatin exposure of sensitive cells has been previously associated with a down-regulation of Bcl-x_L expression and apoptosis, whereas recurrence was systematically observed when Bcl-x_L expression was maintained. Bcl-x_L down-regulation could thus constitute an interesting chemosensitizing strategy. We showed that a Bcl-x_L targeted RNA interference strategy efficiently sensitized chemoresistant ovarian carcinoma cells to cisplatin, but some of them were still able to re-proliferate. Considering the possible cooperation between Bcl-x_L and MCL-1, we investigated the possibility to avoid recurrence *in vitro* using a multi-targeted RNAi strategy directed against these two anti-apoptotic proteins. We showed that their concomitant inhibition lead to massive apoptosis in absence of cisplatin, this multi-targeted RNAi approach being much more efficient than conventional chemotherapy. We thus demonstrated that Bcl-x_L and MCL-1 cooperate to constitute together a strong molecular "bolt", which elimination could be sufficient to allow chemoresistant ovarian carcinoma cells apoptosis. Moreover, we demonstrated that in presence of a low concentration of cisplatin, the concomitant down-regulation of Bcl-x_L and MCL-1 allowed a complete annihilation of tumour cells population thus avoiding subsequent recurrence *in vitro* in cell lines highly refractory to any type of conventional chemotherapy. Therefore, Bcl-x_L and MCL-1 targeted strategies could constitute an efficient therapeutic tool for the treatment of chemoresistant ovarian carcinoma, in association with conventional chemotherapy.

Ovarian cancer is the leading cause of death from gynecological malignancies worldwide and the fifth most common cause of cancer death in women.¹ Early diagnosis is difficult owing to the asymptomatic character of this disease in early stages,

and more than 70% of these cancers are diagnosed in an advanced stage (FIGO stages III or IV). Patients with advanced ovarian cancer are treated initially with optimal debulking surgery and standard chemotherapy (platinum-drugs usually associated to taxanes).² Despite an initial 70–80% response rate, most patients will relapse within 1–2 years and develop resistance to chemotherapy³ and the overall 5-year survival is less than 30%. The identification of new drugs or novel therapeutic strategies able to (re)sensitize ovarian carcinoma cells to existing chemotherapy thus appear as a major challenge.

Cisplatin, a DNA-damaging agent that forms DNA adducts, is commonly used for the treatment of advanced ovarian cancers. It is widely accepted that these adducts lead to a cell cycle arrest followed by the induction of apoptotic cell death.⁴ However, it should be considered that in resistant cells, a strong protection against apoptosis exist when the DNA damages reach their maximum level, to allow the repair of these injuries and the recovery of a normal proliferation. As a result, activation of anti-apoptotic pathways seems to be essential for the survival of damaged tumour cells, at least during the few days following cisplatin exposure.

Bcl-2 protein family appears as critical for apoptosis commitment, in particular *via* the control of the mitochondrial

Key words: apoptosis, Bcl-2 family, Bcl-x_L, MCL-1, cisplatin

Abbreviations: CDDP: cisplatin [cis-diamino-dichloro-platinum (II)]; DAPI: 4',6-diamidino 2-phenylindole; PI: propidium iodide; siRNA: small interfering RNA; 5-FU: 5-fluorouracil

The first two authors contributed equally to this work

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Correspondence to: Laurent Poulain, Groupe Régional d'Etudes sur le Cancer, Unité "Biologie et Thérapies Innovantes des Cancers Localement Agressifs", Centre de Lutte Contre le Cancer F. Baclesse, 3 Avenue du général Harris, 14076 Caen cedex 05, France, E-mail: l.poulain@baclesse.fr