HUMAN LUNG CARCINOMA SENSITIVITY TO PACLITAXEL : WHICH ROLE FOR BIM ?

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INTRODUCTION

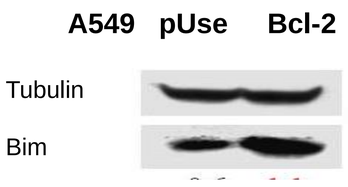
Apoptosis is defined as mechanism of cellular suicide involved in the regulation of tissue homeostasis. Its deregulation is one of the causes of cancer development. Bcl-2 family members are central regulators of apoptosis. They are subdivided into two classes, the proapoptotic members (which include Bim) and antiapoptotic members (like Bcl-2).[1] The overexpression of Bcl-2 is generally associated with many cancer and resistance to chemotherapy, including microtubule-targeting agents (MTAs).[2] Therefore several anti-Bcl-2 strategies are in development.[3] Unexpectedly, several studies show that a decrease of Bcl-2 may be associated with resistance to MTAs. This paradoxical role of Bcl-2 has not found a clear explanation yet. This study shows that overexpression of Bcl-2 leads overexpression of Bim, which is responsible of an increasing sensitivity to MTAs. Bim is a potential biomarker which may be include in predictive test to determine the reponse to paclitaxel treatment in human lung carcinoma. Our work also allows a better understanding of Bim's gene regulation.

MATERIALS AND METHODS

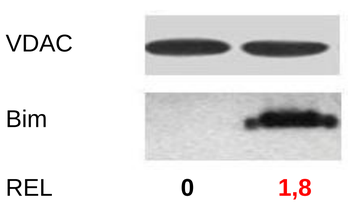
The techniques which are used to study the sensitivity of cells to MTAs are the Western Blot and immunofluorescence. To study Bim's gene regulation, we have used the technique of a reporter gene.

RESULTS

I. OVEREXPRESSION OF THE PROAPOPTOTIC PROTEIN BIM IN A549 BCL-2

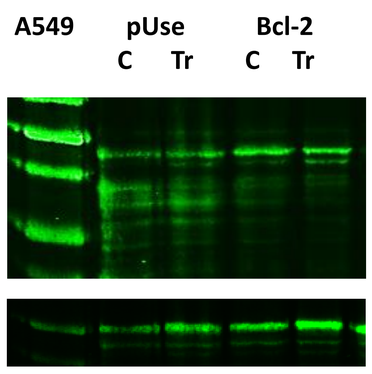


Overexpression of Bim in A549 Bcl-2 at cellular and mitochondrial level



Overexpression of Bim in A549 Bcl-2 at mitochondrial level

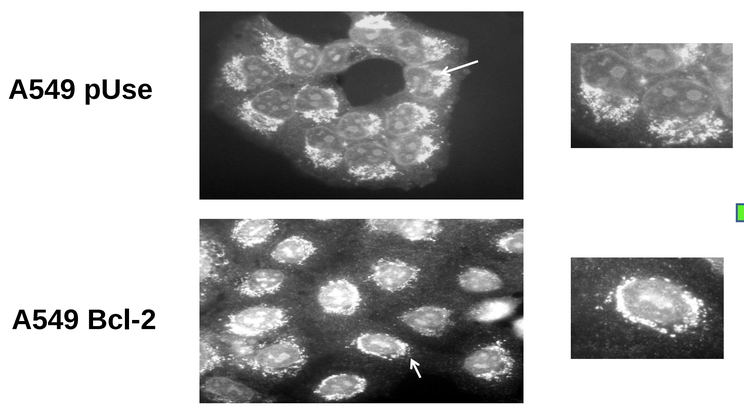
II. ACTIVATION OF APOPTOSIS MITOCHONDRIAL PATHWAY



Bim Up-Regulation Is Responsible for the Enhanced Efficacy of MTAs in Bcl-2-Overexpressing Cells

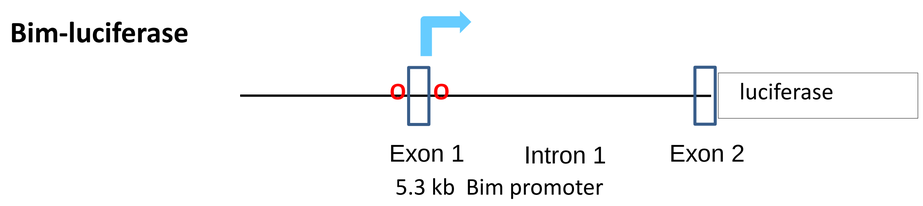
III. FOXO3A LOCALIZATION INTO CELLS A549 pUse AND Bcl-2

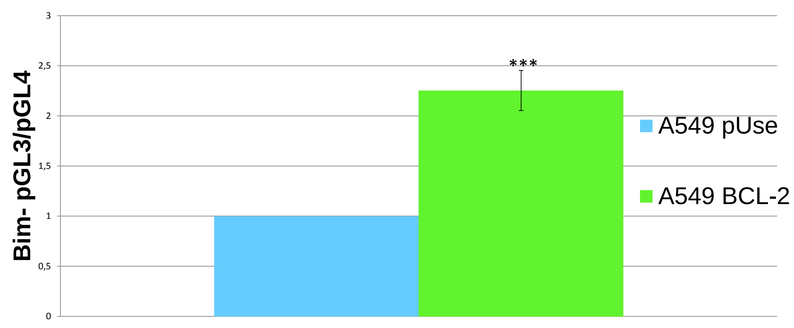
Bim mRNA expression is mainly regulated by FoxO3a, a member of the FoxO family of transcription factors. We first studied FoxO3a localization, because it must reside in the nucleus to bind to its cognate DNA targeting sequences.



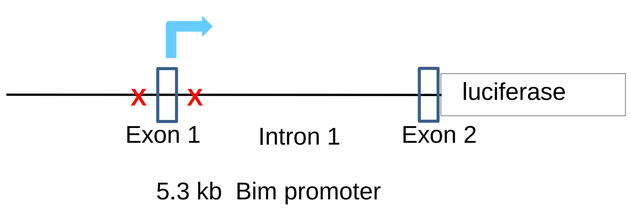
FoxO3a was mainly present in the cytoplasm of A549 pUse cells, while it partly colocalized with the nucleus in A549 Bcl-2 cells

III. BIM EXPRESSION IN BCL-2-OVEREXPRESSING CELLS IS MODULATED BY FOXO3A

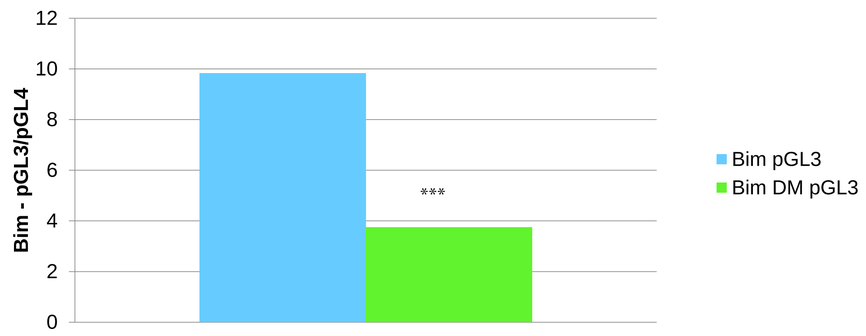




Luciferase activity was measured in A549 pUse and A549 Bcl-2 cells. Bim promoter transcriptional activity was increased 2.3 ± 0.2-fold in A549 Bcl-2 cells *versus* A549 pUse cells (p<0,001)..



A549 Bcl-2 cells were transfected with Bim-pGL3 DM, a double mutant *Bim*-LUC reporter construct that contains mutations in the bim1 and bim2 FoxO sites.



We recorded a 2,7 ± 0,3 fold decrease in *Bim* promoter transcriptional activity (p<0,001) in A549 Bcl-2 cells transfected with a FoxO-mutated binding site *Bim*

DISCUSSION

These data suggest that Bim is a more reliable marker of the sensitivity to MTAs than Bcl-2. Such a predictive test showing the level of Bim expression be able to predict therapeutic efficacy and/or resistance based on molecular profiling of the tumors. However, the induction of Bim only can not be sufficient for a significant cellular death. Indeed, it is more likely that Bim acts in unison with the other pro-apoptotic proteins. So the development of targeted therapies, on the family Bcl-2 in particular, must pass by a better understanding of the molecular mechanism involved in apoptosis regulation.

[1] Borner C. The Bcl-2 protein family: sensors and checkpoints for life-or-death decisions. Mol Immunol. 2003 Jan;39(11):615-47. Review
[2] Yoshino T and al Bcl-2 expression as a predictive marker of hormone-refractory prostate cancer treated with taxane-based chemotherapy. Clin Cancer Res. 2006 Oct 15;12(20 Pt 1):6116-24
[3] Dos Santos LV and al. Bcl-2 targeted-therapy for the treatment of head and neck squamous cell carcinoma. Recent Pat Anticancer Drug Discov. 2011 Jan;6(1):45-57

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