Differential cell cycle control protein profiles characterize bronchial carcinoids sensitive to mTOR inhibitors

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BACKGROUND

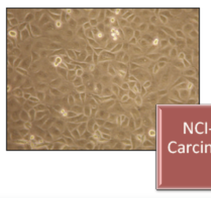
Bronchial Carcinoids (BC) are rare Neoplasm, still orphan of medical therapy, which arise from neuroendocrine cells. It has been previously demonstrated that the atypical BC human cell line NCI-H720 is sensitive to Everolimus (E), an m-TOR inhibitor, in terms of cell viability reduction, with a G0 cell-cycle arrest and a Cyclin D1 protein reduction. On the contrary, the typical human BC cell line NCI-H727 is not sensitive to E, despite the Cyclin D1 reduction. The mechanisms underlying this phenomenon have not been fully clarified, yet.

AIM

Our aim is to further investigate cell cycle mechanisms that underlie resistance to mTOR inhibitors in BC cells, in order to identify new therapeutic approaches.

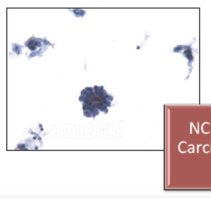
PRELIMINARY DATA

As we have previously demonstrated in our laboratory, Human BC in vitro cell lines, NCI-H720 and NCI-H727, are representative of our in vitro models for BC cell lines SENSITIVE and RESISTANT to Everolimus, respectively.



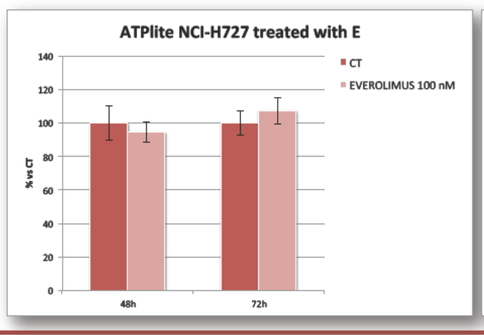
NCI-H727 Typical Carcinoid Human cell line

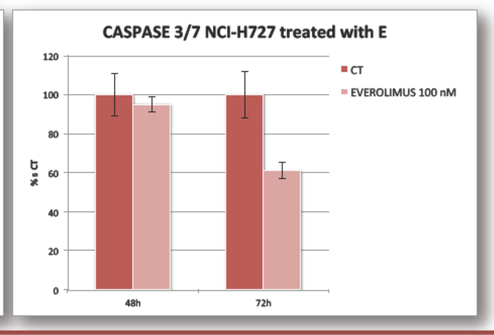
RESISTANT TO RAD001

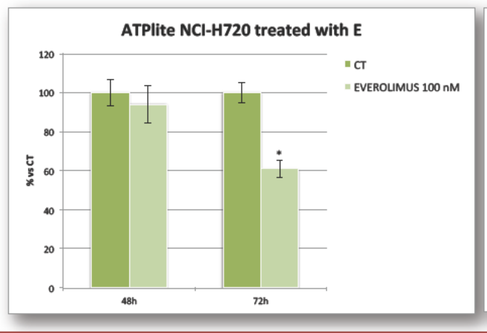


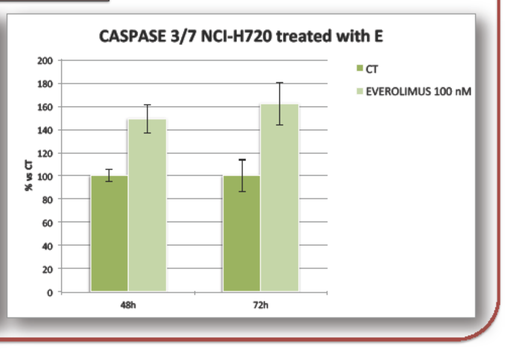
NCI-H720 Atypical Carcinoid Human cell line

SENSITIVE TO RAD001



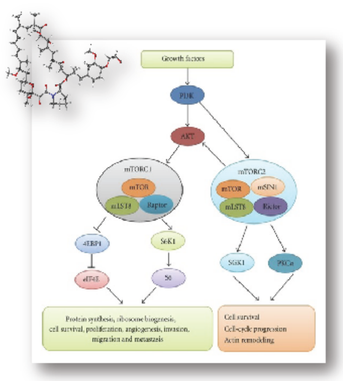






MATERIALS AND METHODS

SUBSTANCE:
EVEROLIMUS, an mTORC1-inhibitor.

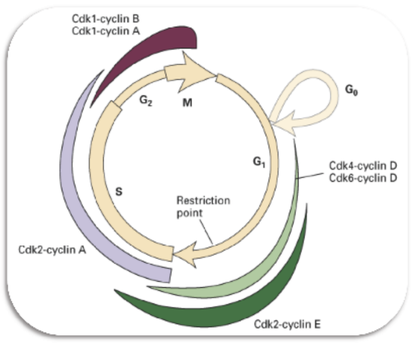


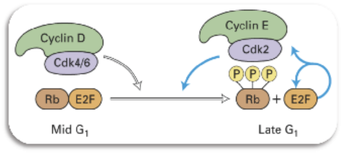
NCI-H720 and NCI-H727:

Human BC cell line cultures were investigated by Western blot, before and after a challenge with E.

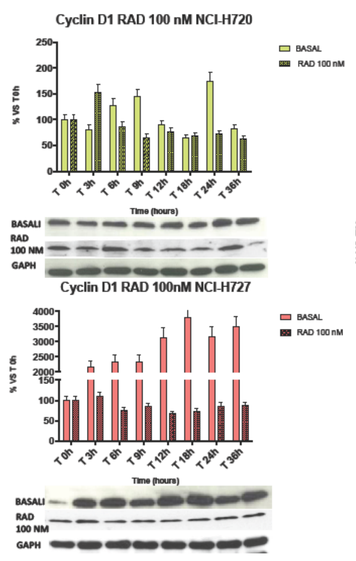
Cell cultures were synchronized with a low-FBS-concentration-Medium (Time 0) and than collected at different time points for WB analysis of several cell-cycle control proteins, such as:

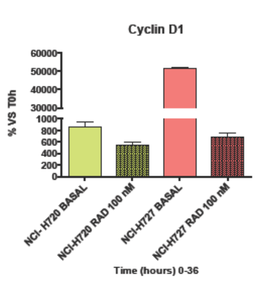
- Cyclin D1/CDK4
- Cyclin E/CDK2
- $p27^{kip1}$/phospho-$p27^{kip1}$ SER10

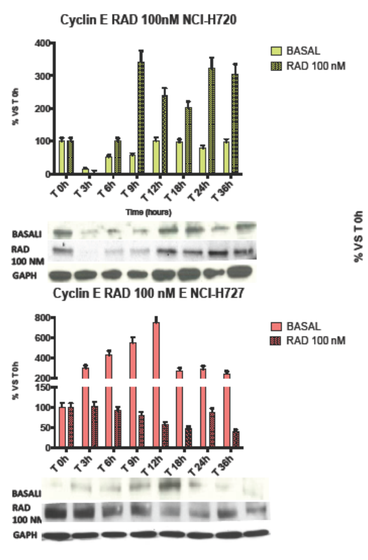


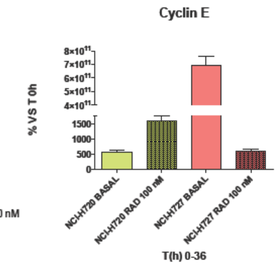


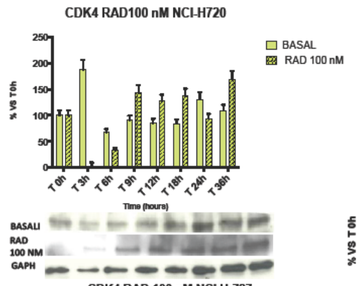
RESULTS

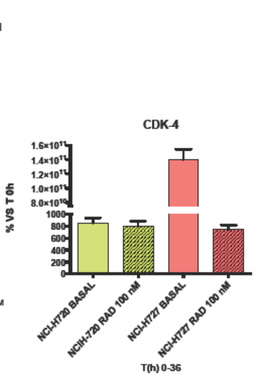


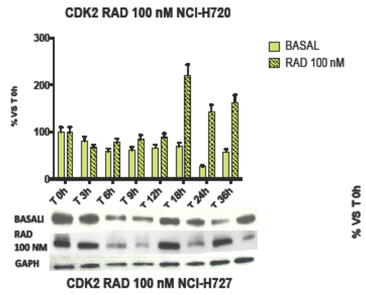


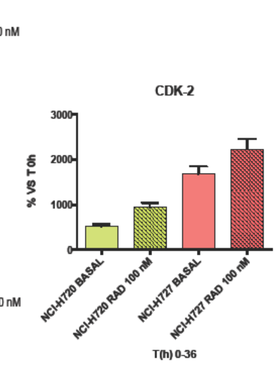


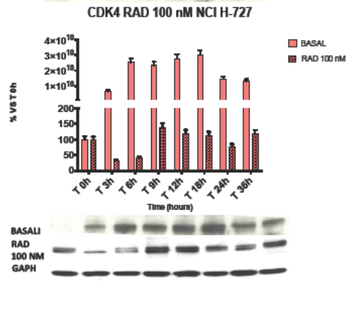


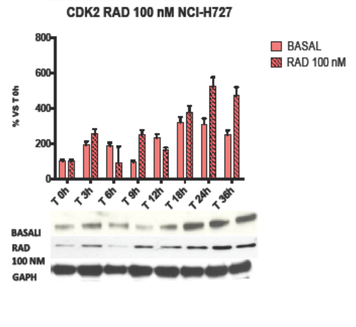


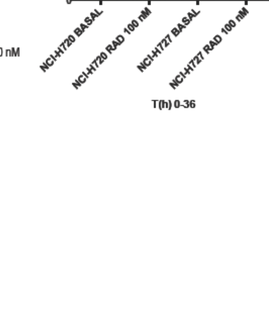












THE TWO BC CELL LINES SHOW DIFFERENT BASAL LEVELS OF PROTEIN COMPLEX CYCLIND1/CDK4 AND CYCLIN E/CDK2.

THE TYPICAL BC CELLS SHOW AN HIGHER PROTEIN LEVELS EXPRESSION OF THIS TWO COMPLEXES SUGGESTING A CELL CYCLE PROGRESSION ACCORDING TO THE AGGRESSIVE PHENOTYPE ASSOCIATED TO THE BC CELL LINE NCI-H727.

IN THE SENSITIVE BC CELLS (NCI-H720), TREATMENT WITH E, REDUCE CYCLIN D1/ CDK4 PROTEIN LEVELS AND INDUCE THE CYCLIN E/CDK2 SUGGESTING THAT OTHER MECHANISMS ARE INVOLVED IN G0 ARREST PREVIOUSLY OBSERVED.

IN THE RESISTANT BC CELLS (NCI-H727), TREATMENT WITH E, INDUCED A REDUCTION IN CYCLIN D1/ CDK4 AND CYCLIN E PROTEIN LEVELS BUT NOT CDK2.

BASAL LEVELS OF $p27^{KIP1}$ AND PHOSPHO p27 SER10 ARE HIGHER IN THE RESISTANT BC CELL LINE AS COMPARED TO THE SENSITIVE ONE, SUGGESTING A REDUCTION IN THE INHIBITORY FUNCTION OF $p27^{kip1}$;

AFTER TREATMENT WITH E A SIGNIFICANT REDUCTION IS OBSERVED ONLY IN THE RESISTANT BC CELL LINE SUGGESTING AN INVOLVMENT OF $p27^{kip1}$ IN THE HUMAN BC CELLS RESPONCE TO (E)

CONCLUSIONS

Our data indicate that resistance to mTOR inhibitors may be linked to a deranged cell cycle control protein profile. Therefore the characterization of these proteins may represent a putative marker of resistance to E, possibly contributing to a better patients selection for a therapeutic approach with mTOR inhibitors.

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Reproduction, endocrine disruptors and signalling
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