**DIFFERENTIAL CELL CYCLE CONTROL PROFILES CHARACTERIZE BRONCHIAL CARCINOIDI SENSITIVE TO mTOR INHIBITORS**

**Benfini Katiuscia**, Gagliano Teresa, Gentilini Erica, Riva Eleonora, Falletta Simona, Di Pasquale Carmelina, Bondanelli Marta, Ambrosio Maria Rosaria, degli Uberti Ettore, Zatelli Maria Chiara.

Department of Medical Science, Section of Endocrinology and Internal Medicine University of Ferrara, Italy

**BACKGROUND**

Bronchial Carcinoids (BC) are rare Neoplasms, 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.

**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 then collected at different time points for WB analysis of several cell-cycle control proteins, such as:

- Cyclin D1/CDK4
- Cyclin E/CDK2
- p27Kip1/phospho-p27Kip1 SER10
- p27

**RESULTS**

The two BC cell lines show different basal levels of protein complex cyclin/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, reduces cyclin D1/CDK4 protein levels and induces 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 p27Kip1 and phospho-p27SER30 are higher in the resistant BC cell line as compared to the sensitive one, suggesting a reduction in the inhibitory function of p27Kip1.

After treatment with E a significant reduction is observed only in the resistant BC cell line suggesting an involvement of p27Kip1 in the human BC cells response 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.