P40 Cross-resistance to Cabozantinib in renal cell carcinoma second-line treatment?

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Introduction & Objectives: Owing to its heterogeneity and up regulation of survival intracellular signaling, metastatic renal cell carcinoma (mRCC) often resists to the standard first-line treatments, the tyrosine kinase inhibitors (TKIs). It is poorly unraveled how the long-term administration of the TKI affects the response of tumor cells undergoing a second cycle of TKI treatment. Hence, we studied *in vitro* whether RCC cells develop cross-resistance to the multi-targeted TKI Cabozantinib (Cbz), frequently used in the second-line setting. Moreover, we tested if the direct inhibition of its main target, the oncogene Met, might alter intracellular pathways of the RCC resistant cells.

Materials & Methods: We evaluated the *in vitro* effect of Cbz on previously generated Sunitinib-resistant cells (786-O-Suni) in comparison to their untreated counterpart (786-O-WT). Drug sensitivity was determined via WST-1 cell proliferation assay, whereas the effect of Cbz on cell pathways was evaluated after 8, 24 and 72h by means of western blotting analysis.

Results: The sensitivity of the 786-O-WT to Cbz was assessed at IC50 value of 9.9 μM. Challenging 786-O-WT with Cbz at different incubation times reduced the expression of the receptor Met and its downstream intracellular pathways: the activated Src (pSrc) and p38 MAPK (p-p38 MAPK). A significant down regulation of these kinase proteins was reported after 8h treatment. Likewise, Erk protein showed a slight increased activity (pErk) after 8 and 24h drug exposure. In contrast, 786-O-Suni cells were less sensitive to Cbz (IC50:12.2 μM) with no significant changes in MET, nor pSrc and p-p38 MAPK kinase expression. However, pErk showed time-dependent up regulation following Cbz treatment. Finally, phosphorylated S6 (pS6) was used as a marker of PI3K/Akt/mTOR pathway. Cbz decreased the level of pS6 in 786-O-WT, while boosting it in the 786-O-Suni cells.

Conclusions: Our initial *in vitro* data confirmed Cbz as a potent tool to contrast RCC progress in the first-line setting. Still, resistance onset during Sunitinib treatment might be linked to acquired cross-resistance to Cbz. Moreover, our evidence suggests that Cbz might drive overlapping survival mechanism, as the drug did not affect the Met-downstream pathways in 786-O-Suni cell line. This ongoing work will provide a better knowledge of the molecular patterns of possible cross-resistance in RCC by analyzing additional cell lines including Pazopanib-resistant ones.