# Extracellular matrix remodeling and invasive potential but not epithelial-to-mesenchymal transition markers are targeted in vitro in renal cell carcinoma cells by Ukrain administration

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Renal cell carcinoma (RCC) is the most common malignancy of the kidney, arising from the cortical tubular epithelium and accounting for 85% of renal cancers. Recent clinical investigations suggested that Ukrain (UK), a semisynthetic derivative of the greater celandine alkaloids derivatized with thiotepa, exerts beneficial effects in the treatment of several solid tumors, including the RCC. In this study we investigated whether UK was able to modulate the malignant phenotype of RCC cells, by analyzing the expression of proteins involved in tumor progression.

Caki-1, Caki-2, and ACHN cell lines derived from RCC were treated or not (CT) with three doses of UK (5, 10 and 20 μM) for 48h. Epithelial-to-mesenchymal transition (EMT) markers such as E-cadherin, beta-catenin and vimentin were analyzed by immunofluorescence; matrix metalloproteinase (MMP)-2 and -9 activity was analyzed by SDS-zymography. Intracellular and secreted levels of Secreted Protein Acidic and Rich in Cysteine (SPARC) were determined by Western blot, and cell cycle analysis by flow cytometry.

UK was not able to induce E-cadherin and beta-catenin immunoreactivity on cell membrane as well as to modify vimentin distribution. By contrast, MMP-2 activity was significantly down-regulated after 20 μM UK administration in all cell lines; a similar pattern was observed for MMP-9 activity in Caki-1, whereas in Caki-2 and ACHN MMP-9 decrease was not statistically significant. Since both MMP-2 and MMP-9 seem to be engaged in the promotion and progression of RCC, their down-regulation in UK-treated cells suggests that the drug may modulate the invasive potential of RCC.

The overall pattern of SPARC expression shows that UK is able to strongly down-regulate its secretion in cell supernatants at all UK doses in Caki-2, and at 20 μM UK in Caki-1 and ACHN cells, suggesting that UK may also inhibit extracellular matrix remodeling of the tumor microenvironment, possibly rendering tumor microenvironment less permissive for tumor invasion. At the same time, an up-regulation of intracellular SPARC protein levels was observed in Caki-1 and ACHN (p ns), and in Caki-2 cells ($p<0.05$ for CT vs 20 μM UK), suggesting that UK could also affect cell proliferation by cell cycle inhibition, as supported by the cell cycle analysis, since SPARC exerts also a role as cell cycle inhibitor.

Considered as a whole, our results suggest that UK may exert some effects on two major aspects involved in RCC progression, such as tumor invasion/microenvironment remodeling and, possibly, cell proliferation.