The cell-autonomous and non-cell autonomous activities of the alarmin-like RNASET2 protein in prostate cancer models.

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Molecules making tumour cells visible by the immune system represent a promising tool in tumour immunology. In this context, alarmins, defined as stress-induced secreted molecules of endogenous origins acting as early warning signals for the immune system, represent a relevant example. RNASET2, a highly conserved human extracellular RNase endowed with a powerful oncosuppressive activity, has been recently reported to behave as an alarmin-like factor.

Here, we investigated the activity of RNASET2 overexpression *in vitro* and *in vivo*, together with the subsequent effects on macrophage polarization, in *in vitro* and *in vivo* models of prostate cancer PCa. RNASET2-overexpressing PC-3 and 22Rv1 prostate cancer cell lines, generated by transfection with a RNASET2 expression vector or a control empty plasmid, were used to assess cell proliferation, colony formation, adhesion, and migration, coupled to FACS analysis for molecules involved in migration/invasion (CXCR4, CXCL12), angiogenesis (VEGF, CXCL8) and inflammation (TNFα, IFNγ). *In vivo* studies were carried out in nude mice subcutaneously injected with *RNASET2*-overexpressing PC-3 or 22Rv1 cells, to evaluate tumour cell growth, tumour weight and M1/M2-like macrophage infiltration and ratio.

RNASET2-overexpressing 22Rv1 cells showed a reduced capability to proliferate and to generate colonies *in vitro*, compared to RNASET2 overexpressing PC-3 cells. *RNASET2* expression also affected 22Rv1 and PC-3 cells ability to produce factors involved in migration/invasion (CXCR4, CXCL12), or angiogenesis (VEGF, CXCL8). Moreover, a marked decrease in their ability to produce pro-inflammatory cytokines (TNFα, IFNγ) was observed. RNASET2 overexpression affected the cytoskeleton organization as well, as previously reported in other cancer cell lines.

Finally, mice injected with RNASET2-overepressing 22Rv1 cells showed a decrease tumor growth rate coupled toincreased intratumoral M1-like macrophages infiltration, while reducing M2-like macrophages, as compared to those receiving control 22Rv1 cells. The same *in vivo* effect was not observed with PC-3 cells.

Collectively, our results suggest a cell line-specific role of RNASET2 in different PCa models, as a molecule able to act both in a cell autonomous and non-cell autonomous mechanism.