Selective modulation of TNF receptor 2 (TNFR2) promotes renal cancer stem cell survival

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Abstract

Tumour necrosis factor-alpha (TNF) via TNFR2 may act as an autocrine growth factor for tumour cells in renal cell carcinoma (RCC) [1]. Clear cell RCC (ccRCC), the most prevalent form of RCC, contains cancer stem cells (CSC) that are positive for CD133 (RCC-CD133$^+$), which give rise to progeny that form the bulk of the tumour. CSCs are rarely in cell cycle and, as non-proliferating cells, resist most chemotherapeutic agents. Thus, recurrence after chemotherapy may result from the survival of CSCs. Therapeutic targeting of both CSCs and the differentiated tumour populations may provide a more effective strategy for ccRCC treatment.

We have previously reported that TNF via TNFR2 signalling causes quiescent RCC-CD133$^+$ cells to enter cell cycle, rendering them susceptible to cell-cycle-dependent chemotherapeutics [2]. In this study, we determined signalling pathways activated by treating RCC-CD133$^+$ CSCs or non-tumour kidney stem cells (NK-CD133$^+$) in cell culture and as well as in human organ cultures of ccRCC vs adjacent non-tumour kidney (NK) with TNF (wtTNF) or TNF muteins selective for TNFR1 (R1TNF) or TNFR2 (R2TNF) [3].

Immunofluorescence microscopy of tissue and flow cytometry of cultured RCC-CD133$^+$ cells indicate that wtTNF or R2TNF induce serine (pStat3^{S727}) but not tyrosine phosphorylation of Stat3 (signal transducer and activator of transcription 3) and that pStat3^{S727} colocalises with mitochondria. wtTNF or R1TNF, but not R2TNF induced cell death, assessed by TUNEL. These responses appear to be greater in RCC-CD133$^+$ than in NK-CD133$^+$. Confocal microscopy also revealed that increased expression of TNFR2 and pStat3^{S727} induced by R2TNF was primarily found in proliferating RCC-CD133$^+$ cells, indicated by pH3 (anti-phospho-Histone H3) nuclear staining. Knockdown of TNFR2 or of Stat3 by siRNA caused cell death (detected by TUNEL), which was more extensive in RCC-CD133$^+$ cells (49$\pm$1.5%) compared to NK-CD133$^+$ cells (30$\pm$2.2%). Addition of exogenous wtTNF or R1TNF further increased cell death by a small margin (wtTNF; 58%$\pm$0.9 vs 42%$\pm$0.32 and R1TNF; 54%$\pm$1.1 vs 38$\pm$1.0%). Similar findings were observed in organ cultures. TNFR2 signalling also led to phosphorylation of mTOR^{S2448}, Akt^{T308}, VEGFR2^{Y1054} and PI3K^{p110ß}.Treatment of organ cultures with specific kinase inhibitors of these phosphorylated proteins (mTOR, Akt, PI3K and VEGFR2) prior to TNF treatment resulted in diminished levels of proliferating cells and also increased cell death. In conclusion, our new data suggest that TNFR2 signalling increases cell survival as well as cell cycle entry in both normal and malignant CSCs and further suggests that the survival signal may involve serine phosphorylation of Stat3 and recruitment to mitochondria.

References

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