

Validation of BRCA1 associated protein-1 (BAP-1) as an adverse prognostic factor and investigations into the impact of BAP1 loss on the vascular endothelial growth factor (VEGF) pathway in clear cell renal cell carcinoma (ccRCC).

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Introduction&purpose:

Loss of BAP1 has been described as an adverse prognostic factor in ccRCC. Therefore, the current study aims to validate *BAP1* loss as an adverse prognostic factor and to investigate the impact of BAP1 loss on the VEGF pathway in ccRCC.

Methods:

The study cohort was comprised of 321 ccRCC patients of whom 294 had evaluable tissue micro array (TMA) data. Patients with vs. without loss of BAP-1 expression were compared for differences in features, survival outcome and correlations with expression of the VEGF pathway. Hypoxia inducible factor (HIF)-1 α and HIF-2 α levels as well as the VEGF-pathway members VEGF-A, VEGFR1, and VEGFR2 were analyzed with WB and enzyme linked immunosorbent assay (ELISA) in normoxic (21%O₂) and hypoxic (1% O₂) BAP1 negative (UMRC6), BAP1 mutated (mt) (769-P) and BAP1-wild type (WT) (RCC-JF) ccRCC cell lines over a time period of 72hours.

Results:

In the TMA analysis, patients with vs. without BAP1 loss had significantly advanced clinicopathological features (higher T stages (p<0.001), M1 (p=0.017), larger tumors sizes

($p=0.003$), sarcomatoid features ($p=0.018$)). BAP1 loss was associated with worse survival outcome for both cancer specific (CSS) ($p < 0.001$) and recurrence free survival (RFS) ($p < 0.001$). However, when adjusted, BAP-1 loss could not be validated as an independent prognostic factor of either CSS ($p=0.183$) or RFS ($p=0.233$). Correlations with members of the VEGF pathway demonstrated high cytoplasmic HIF-1 α ($p=0.048$), VEGF-A epithelial (epi) ($p=0.008$), VEGFR1 epi ($p < 0.001$), VEGFR2epi ($p=0.016$), VEGFR2endo ($p=0.035$), and low expression of VEGFR3 ($p=0.037$) in BAP (-) vs. BAP1 (+) tumor tissue.

BAP1 was found in 769-P, RCCJF in both the cytoplasm and nucleus while BAP1 was absent in UMRC6. In (BAP-1 (-)) cells, HIF-1 α levels increased to 10-100 fold and 40-250 fold higher levels in the cytoplasm and the nucleus under hypoxic and normoxic conditions when compared with the RCC-JF (BAP1 WT) and 769-P (BAP1 mt) cell lines at 24-72h. In the BAP1-WT and BAP1-mt cell lines, HIF-1 α levels were absent in the cytoplasm and were constant in the nucleus during the observation period. Similarly, VEGFR2 levels were 8-14 fold higher under both normoxic and hypoxic conditions in the BAP1 (-) cells during the observation period while VEGFR1 levels were comparable in all three cell lines. Interestingly, BAP1(-) and BAP1-mt cell lines presented a 3-5 fold higher extracellular/cellular VEGFA ratio when compared with BAP1-WT cell lines.

Conclusions:

The current study has validated that BAP1 loss is associated with adverse prognostic tumor features and poor prognosis in ccRCC. Additionally, our TMA and *in-vitro* results suggest a potential role of BAP1 in the regulation of the VEGF pathway.