Identification of tumorigenic cells and therapeutic targets in pancreatic neuroendocrine tumors

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Patients with pancreatic neuroendocrine tumors (PanNETs) have limited therapeutic options. Tumor initiating cells (TICs) are responsible for tumor development, metastasis, and recurrence. Targeting TICs is necessary to eradicate tumors. TICs remain uncharacterized in PanNETs. MET is principal regulator of PanNET aggressiveness in mouse models and cell lines. Patients with pancreatic neuroendocrine tumors (PanNETs) have limited therapeutic options.

**Introduction**

We performed an in-depth genomic, cell surface, and functional analysis of a PanNET tumor at different stages in the disease process from a single index patient. We then used 39 separate human tissue specimens (IRB Protocol 22185) to develop a novel PanNET cell line (APL1) and generate reproducible xenograft models. We profiled patient PanNET tumors using flow cytometry, immunostaining, RNAseq, gene-set enrichment analysis, proteomic profiling, and tissue microarray analysis to identify a tumorigenic population of cells and potential therapeutic targets. We used in vitro phagocytosis assays using BON and APL1 cells co-cultured with human donor or NOD scid gamma (NSG) mouse macrophages to screen potential immunotherapies. We used in vivo treatment experiments using NSG-xenograft or RIP-Cre Rbp53/p130 mice to validate potential immunotherapies.

**Methods & Materials**

Profiling of an index patient’s PanNETs (a). Gene expression analysis of the primary tumor and normal pancreas (b-g) shows aberrant expression of HGF and MET (b) and CD47 (c). Staining of CHGA and MET in the liver met (b) and LN met (d). Staining of CHGA and HGF in the liver met (j) and LN met (k). Staining of CHGA and HGF in normal liver (l) and pancreas (m). Staining of CHGA and CD47 in the liver met (n) and lymph node met (o).

**Results**

CD47 characterizes distinct cell populations by flow cytometry (a-b). ALDHA1 activity is enhanced in CD90pos vs. CD90neg cells (e). TIC markers are differentially expressed in CD90pos and CD90neg populations (d). Gene set associated with stem cells are enriched in CD90pos vs. CD90neg cells (e-j).

Figure 4. CD90 characterizes distinct cell populations by flow cytometry (a-b). ALDHA1 activity is enhanced in CD90pos vs. CD90neg cells (e). TIC markers are differentially expressed in CD90pos and CD90neg populations (d). Gene set associated with stem cells are enriched in CD90pos vs. CD90neg cells (e-j).

Figure 5. FACS-purified CD90pos cells develop into tumors with high efficiency in NSG mice but CD90neg cells do not (a-c). CD90pos have sustained tumor growth but CD90neg cells do not (d). CD90pos have increased tumorigenic potential compared to CD90neg and unsorted cells (e). Xenografts from CD90pos cells recapitulate the features of the primary patient tumor (f-m).

Figure 6. Proteomic profiling of 332 antigens in 2 cell lines and 4 primary tumors reveals potential therapeutic targets (top 9 are shown). CD47 is highly expressed on all PanNET cells by flow cytometry (b) and immunofluorescence (c). CD47 is co-expressed with MET (d). CD47 expression is enhanced in the CD90pos (TICs) compared to CD90neg cells (e). Kaplan-Meier analysis of tissue microarray staining show that increased CD47 expression is associated with decreased survival compared to decreased CD47 expression (f). High-throughput phagocytosis assays were used to validate potential therapeutic targets (g-h). In-vitro phagocytosis of PanNET cells by macrophages is enhanced with anti-CD47 and anti-CD99 therapies as well as cetuximab (c-e).

Figure 7. Anti-CD47 therapy initiated 2 weeks after primary patient heterotopic xenograft engraftment in NSG mice inhibits tumor growth (a-e) prolongs survival (f) compared to controls.

Figure 8. Anti-CD47 therapy initiated on postnatal day 35 in RIP-Cre Rbp53/p130 mice prolongs survival (a) and inhibits tumor growth (b-c) compared to controls.

Figure 9. Anti-CD47 therapy initiated 2 weeks after primary patient heterotopic xenograft engraftment in NSG mice inhibits tumor growth (a-e) prolongs survival (f) compared to controls.

Conclusions

- MET activation is critical for tumor growth in mouse xenograft models
- CD90 characterizes TICs in PanNETs
- Proteomic profiling reveals potential therapeutic targets that were screened with in vitro phagocytosis assays
- Anti-CD47 therapy inhibits tumor growth, prolongs survival, and prevents metastases in vivo
- Anti-CD47 therapy may synergize with other immunotherapies

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