Stroma-derived secreted factors increase desmoid tumor cell proliferation

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Contains unpublished results
Background

• Communication between tumor cells and the surrounding stroma is an important factor in disease development.

• A major cellular component of the stroma are fibroblasts. But desmoid tumor (DT) cells are also fibroblastic cells.

• Deep sequencing studies show presence of non-mutant populations within “wildtype desmoid tumors.” Also supported by lineage tracing experiments in mice.

• Distinguishing between the two populations allows us to study cell-cell communication, and to reduce variability among samples which can mask important results.
Aims

1. Isolate mutant and non-mutant populations from heterogeneous samples by clonal expansion.  
   \(\rightarrow\) reduce variability

2. Compare surface proteins for potential markers.  
   \(\rightarrow\) improve sensitivity; accelerate the isolation process; info on receptors

3. Compare soluble proteins for potential cell-cell signalling.  
   \(\rightarrow\) identify targetable signaling pathways.
Single cell-derived colonies isolate mutant and non-mutant populations from heterogeneous desmoid tumors

Panel A: We sequenced 14 desmoid tumor tissue samples and found that there is a variability in the mutant cell content within each sample. On average, about half of the cells within a sample do not carry the mutation that caused the tumor. The mutation occurs in the CTNNB1 gene which codes for “beta-catenin”

Panel B: We isolate single cells from these samples, expanded them in culture, and sequenced their DNA. We found that we can identify the non-mutant (e.g. A B C) and mutant (e.g. D E F) populations using this method.

Panel C: Beta-catenin changes the expression of other genes in the cell, including AXIN2. To see if our isolation identifies cells with abnormal beta-catenin activity, we compared AXIN2 levels. We found that the mutant cells indeed have elevated AXIN2 levels, and those levels did not show differences between the specific type of mutation on beta-catenin.

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Surface protein screening identifies CD142 as a mutant-enriched surface marker in desmoid tumors

Panel A: We aimed to find a feature (e.g. a protein on the cell surface) that we can use to distinguish the mutant from non-mutant cells to accelerate our isolation method. We found a protein called “CD142” as one such feature that may define the mutant cells.

Panel B: We also tested other proteins and found CD252 and PDPN as other potential markers. PDPN may define the non-mutant population.

Panel C: We tested the use of “CD142” and “PDPN” in a cell sorting experiment (we scanned cells for whether they have CD142 or PDPN on their surface, then sorted them into two separate “buckets”). We found that this method can separate the mutant from non-mutant cells.

Panel D: We also tested this cell sorting method on a sample that we did not detect a mutation in before (a “wildtype desmoid tumor” sample). We found that we can detect some CD142-positive cells (about 15% of the sample) and these cells do carry a mutation.

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CD142 expression correlates with beta-catenin staining level in desmoid tumors in situ

Panel A: We can use CD142 and PDPN to image cells in tissue to study the location of cells. Here the CD142 cells are colored red and PDPN cells are colored green. We can see that red and green cells co-exist in desmoid tumor tissues. DAPI (blue) is a stain to visualize all cells.

Panel B: We can also see that the proportion of red/green cells changes in different regions of the sample. This coincides with the staining intensity for beta-catenin (the driver of desmoid tumors).

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Non-mutant cell-derived soluble factors increase the proliferation rate of mutant desmoid tumor cells

Panel A: Having separated the mutant and non-mutant populations, we next moved to study what signals these cells are sending to nearby cells. We looked at a panel/array of a number of known proteins that are released/secured from cells that can alter the behavior of neighbouring cells.

Panel B: We evaluated the expression of these secreted proteins and found some (red bars) that are elevated in the mutant cells, and some (blue bars) that are elevated in the non-mutant cells.

Panel C: We found that when the mutant cells are exposed to the proteins released from non-mutant cells, their growth accelerates.

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Non-mutant cell-derived soluble factor, PTX3, phosphorylates STAT6 in mutant desmoid tumor cells

**Panel A:** We conducted another type of panel/array study to see what enzymes inside the mutant cells become activated when they are exposed to the secreted proteins of the non-mutant cells (designated as “CM” here).

**Panel B:** We validated the results we observed in Panel A, and found that one enzyme (STAT6) specifically becomes active.

**Panel C:** We further determined that one of the proteins the non-mutant cells secrete (PTX3) is the one most likely responsible for the activation of STAT6 in mutant cells.

Contains unpublished results
Panel A: When we boil the collection of all secreted molecules coming from non-mutant cells (designated as “WT CM” and “WT CM Boiled” here), we see that the acceleration in growth of the mutant cells is reduced.

Panel B: When we repeat this experiment but instead of boiling the sample we add a drug that inhibits STAT6 activity, we see similar results.

Panel C: Finally, when we repeat this experiment using only PTX3 instead of all of the secreted molecules, we similarly see an increase in the rate of growth of the mutant cells, which we then can prevent by adding the STAT6 inhibitor drug. This means that STAT6 inhibitors have the potential to alter desmoid tumor growth rate, and warrants further testing.
Summary

- Stromal non-mutant fibroblasts secrete soluble factors that can induce the proliferation of mutant desmoid tumor cells (e.g. PTX3/CCL2/CXCL12).
  → Biomarkers? → Variable natural history?

- PTX3 is a stroma-derived soluble factor that increases mutant cell proliferation by activating STAT6.
  → Inflammation? → Drug target?

- Single-cell derived colonies can identify mutant and non-mutant populations within heterogeneous desmoid tumor samples
  → Gene expression profile? Mutation status differences?

- CD142 is a surface protein that is enriched on mutant cells within desmoid tumors.
  → Drug Delivery (ADC)? → Drug target?

Contains unpublished results
Thank You!

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