Effect of PMN Presence on Cancer Vascularization in Colorectal Tumors in Mice

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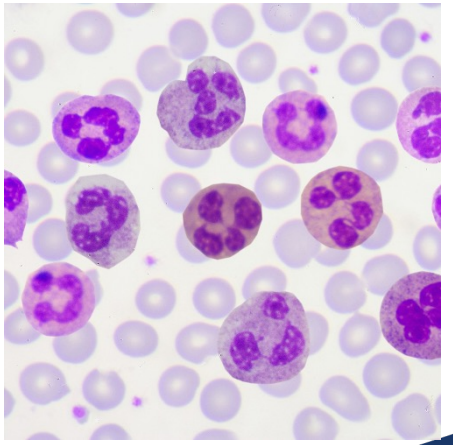
What are PMNs?

- Polymorphnuclear Leukocytes (PMNs) are a type of white blood cells that mainly refers to immune cells like neutrophils
  - The most abundant immune cell (50-60%) and is the first response to foreign pathogens

  or infections
  - Primary function is to kill luminal

  microbes that translocated across

  epithelium and invade mucosa
- Part of the innate immune system



PMNs Involvement in Colorectal Cancer

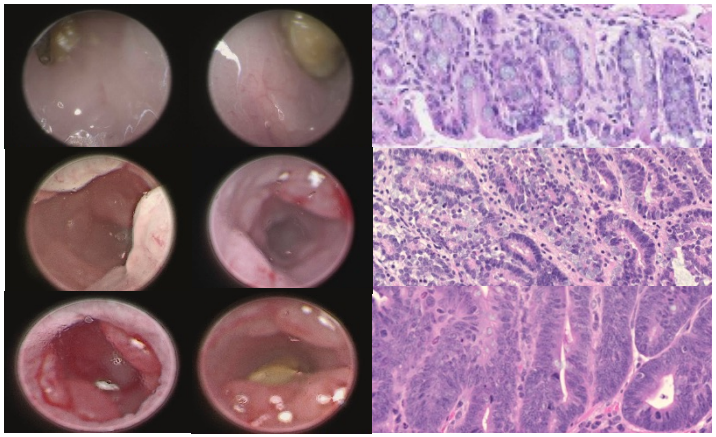
- Ulcerative colitis (UC) is a form of chronic inflammatory bowel disease (IBD), where deregulated immune response promotes exacerbated inflammation and formation of ulcers in the inner lining of the large intestine
  - TANs (Tumor Associated Neutrophils) are a known immune response that exacerbates colorectal cancer through upregulation of MMP, VEGF, and HGF

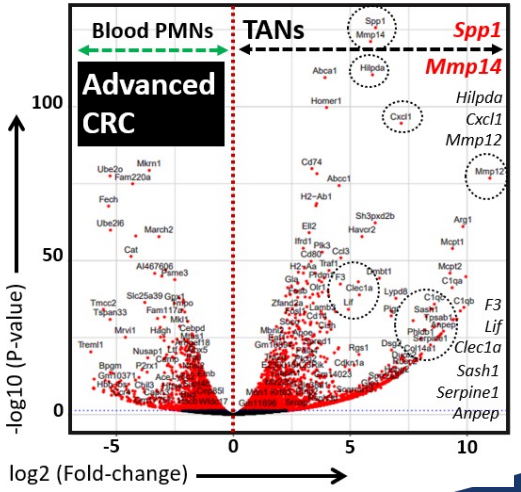
Gene Encoded Proteins

- MMP-14 (metalloproteinase-14)
  - extracellular matrix regulating protein, which has been previously implicated in angiogenesis
  - Codes for matrix metallopeptidase 14
    - Participate in degradation of tissue and is involved in tumor invasion and cell migration
- Spp1
  - Another pro-angiogenic factor
  - Codes for Osteopontin which regulates signaling pathways for cancer progression like immune response and tumorigenesis
- VEGFa
  - Codes for VEGF (Vascular Endothelial Growth Factor)
    - Proliferation and migration of endothelial cells needed for angiogenesis both physiologically and pathologically
- S100A9
  - Is upregulated in colitis colon cancer and other related disorders
  - Established PMN marker

Models and Background

- Tumor infiltrating neutrophils may promote tumor vascularization by providing metalloproteinase-14 and Osteopontin based on AOM/DSS mouse model
  - A model created that encapsulates key factors of human CAC
  - Inexpensive initiation—promotion model
- mRNA sequencing analyses were performed on neutrophils isolated from advance CRC murine tumors.
  - Volcano plot showed top differentially-upregulated genes *Mmp14* and *Spp1*.





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Aim of Research

- Tumor infiltrating neutrophils promote tumor vascularization and growth via the release of metalloproteinase-14 (MMP-14) and Osteopontin.
  - Validate the transcriptomic data of high MMP14 and Spp1 expression in tumor infiltrating neutrophils by Immunohistochemistry (IHC) of advance tumor tissue
  - Establish whether MMP-14 and/or Spp1 directly regulated endothelial cell motility in developing tumors.

Methodology

- Tissue section biorepository from early (low grade) and advanced (high grade) murine colon tumors induced by AOM/DSS treatment (weeks 8 and 14, respectively) with and without prior elimination of tumor neutrophils (PMN^{high} vs PMN^{low}) and stained for MMP-14, Spp1 and VEGFa
  - Qfor expression of these 3 markers in the IHC images and were compared across tumor cross-sections of early vs advanced and PMN^{high} vs PMN^{low} conditions.
  - ImageJ software tracking tool
- Transwell setup was used where endothelial cell migration across collagen coated permeable supports will be induced by introducing recombinant MMP-14 and/or Spp1 to the bottom chamber
  - Optimized appropriate concentration of these recombinant proteins as well as introducing function blocking Abs as positive controls
  - quantification of endothelial cell migration by collecting the transwell filters following 4-6h migration was done, removing all the not migrated cells from the upper side of the filters and by imaging of cells that have migrated to the bottom side

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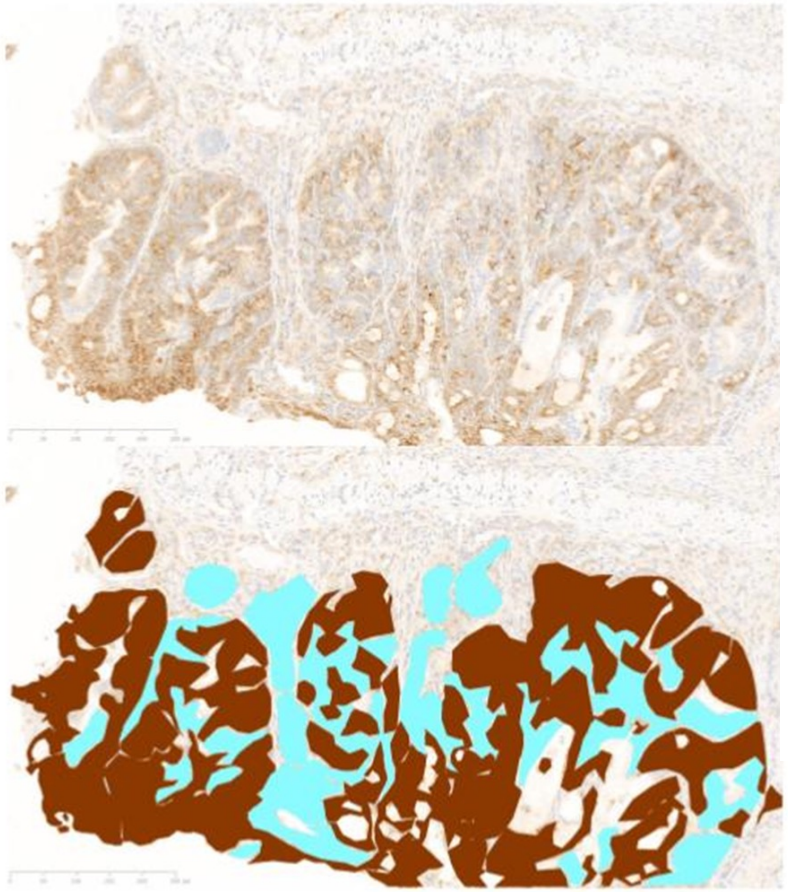

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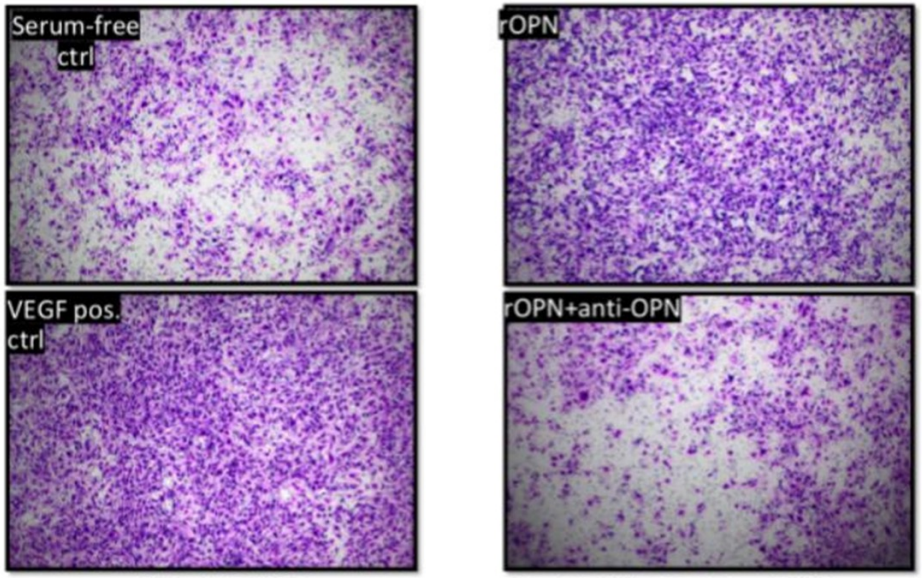

100 ng/mL rVEGF

10µg/mL rOPN; 5.0µg/mL anti-OPN

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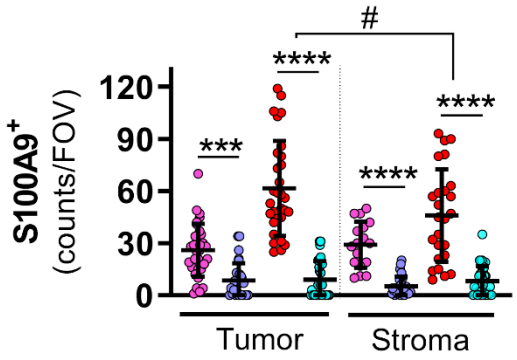

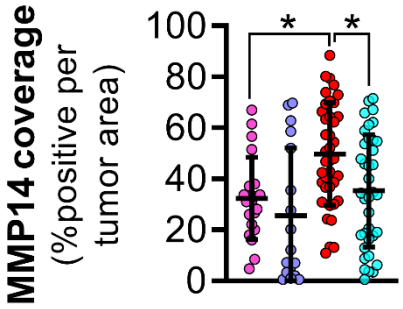

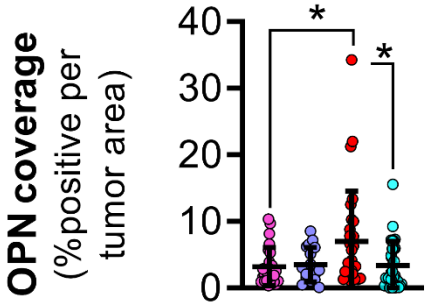

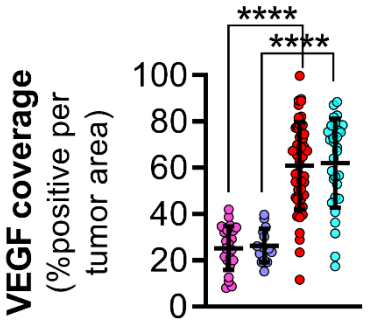

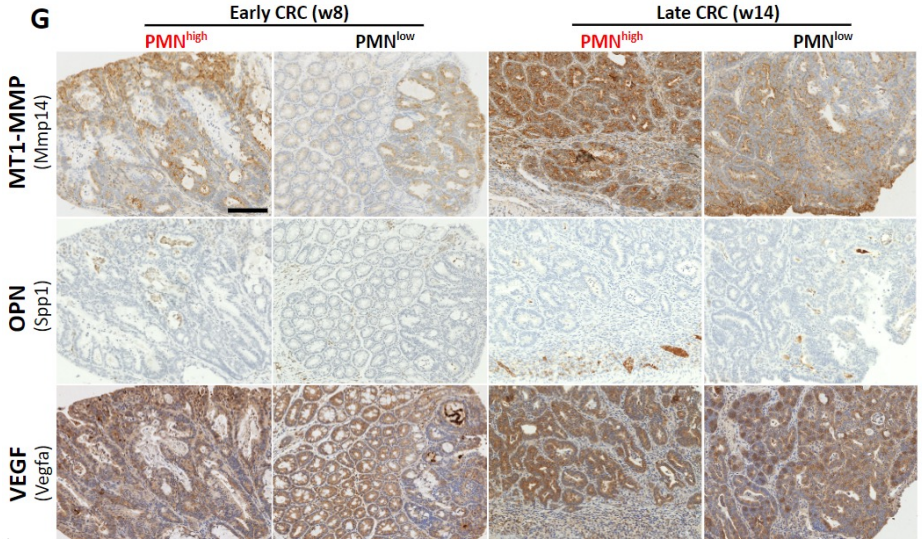

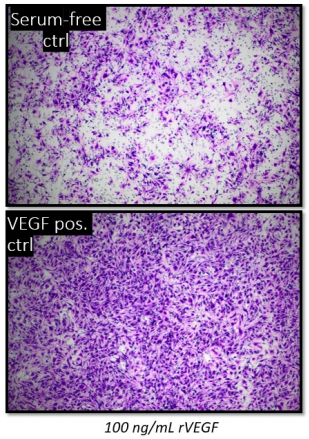

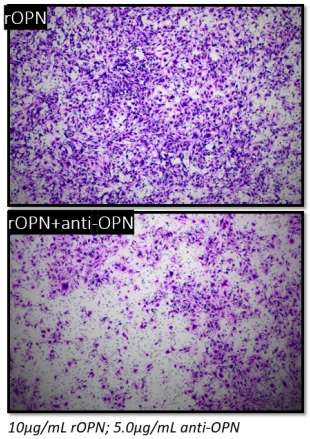

Recombinant OPN:

Increased EC migration

Substrate invasion

Blocking Ab:

Prevent EC migration

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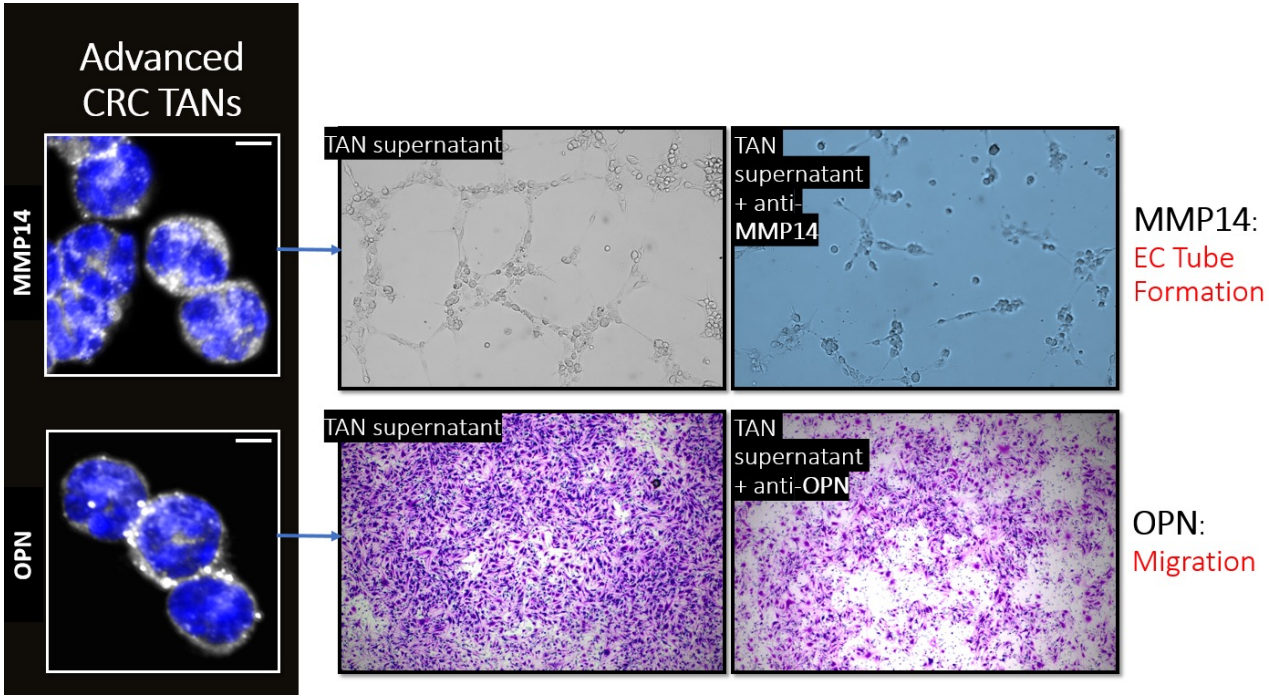
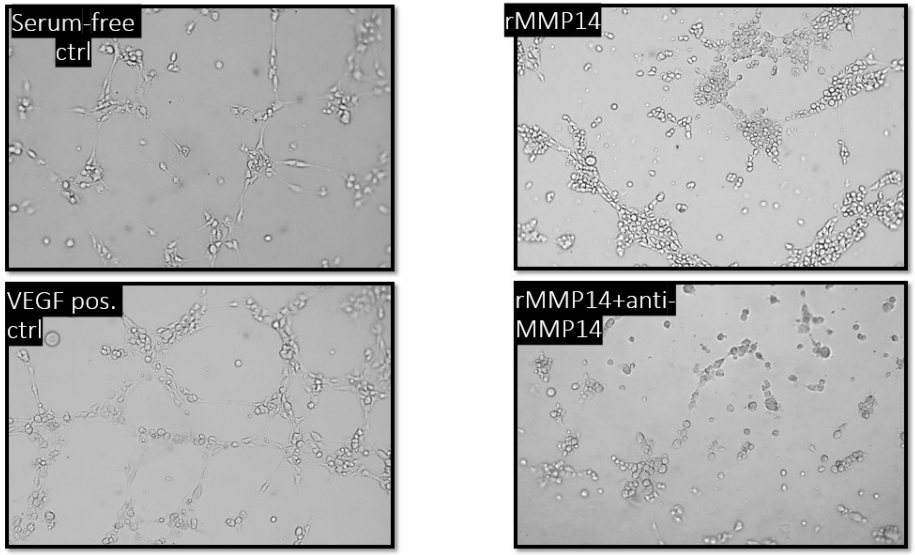


MMP14:
EC Tube Formation

OPN:
Migration



Orderly branching *100 ng/mL rVEGF*

1.0µg/mL rMMP14;10µg/mL anti-MMP14

Recombinant MMP14:

abnormal branching with aggregates

Blocking Ab:

prevent sprouting and branching

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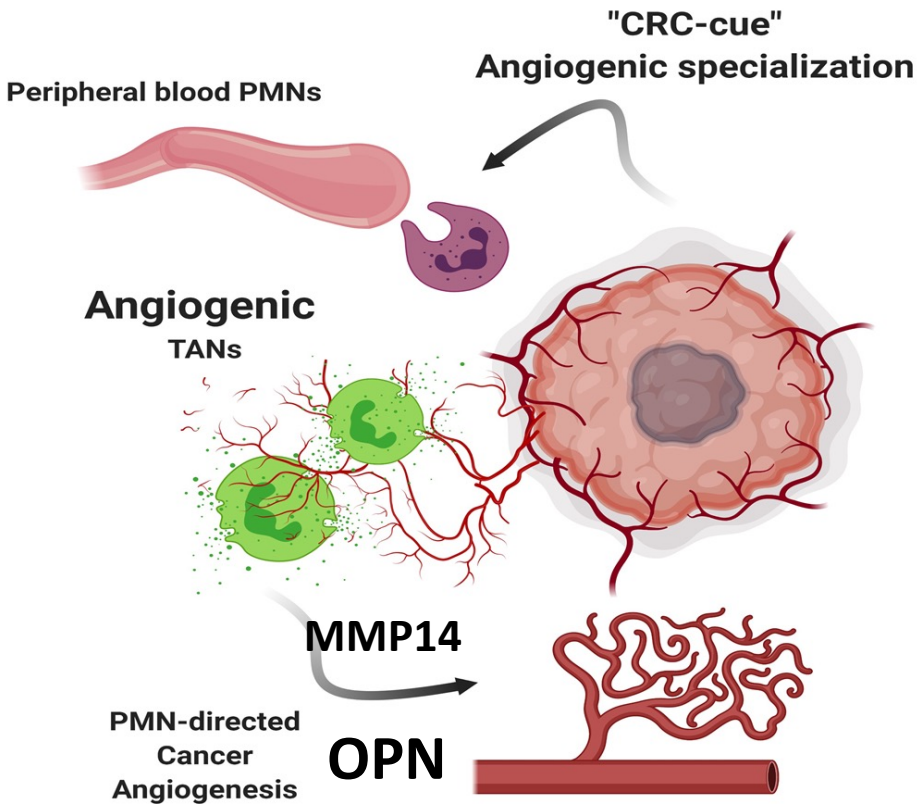

PMNs direct **vessel sprouting and branching** via MMP14 and **guide EC migration** via OPN

How to target?

Small molecule inhibitors

Neutralizing antibodies

Impact on tumor vasculature and tumor burden?

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Discussion

- Through this process, we identified Spp1 (Osteopontin) or MMP14 (MT1-MMP) to be transcriptionally upregulated in PMNs as they entered the cancer environment.
    - In addition, data mining from the National Cancer Institute (NCI) and validation in a separate UC/CRC patient cohort at Northwestern Medicine further confirmed significant upregulation of *Spp1* and *Mmp14* transcripts in high-grade CRC, but not in UC patients.
- The coverages of OPN and MMP14 levels were respectively enriched in the stroma and the tumor center of neutrophil-high tumors during the advanced stages of the disease.
    - This observation indicated that the expression of OPN and MMP14 in the tumor tissues was inducible by the neutrophil presence and could account for the tumorigenic role of these factors in CRC progression.
- Another important angiogenic factor VEGF is however not mediated by the neutrophil presence, but solely enhanced by the progression from early to advanced tumors.
- **On this basis, our analyses showed that tumor infiltrating neutrophils promote tumor vascularization and growth via the release of metalloproteinase-14 (MMP-14) and Osteopontin, contributing to the process of cancer vascularization.**

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