Early events in metastatic spread: new approaches using targeted therapies to disrupt formation of the pre-metastatic niche and development of lung metastases

Khalid A. Mohamadali, Lawrence H. Cheung, and Michael G. Rosenblum

Immunopharmacology and Targeted Therapy Laboratory, Department of Experimental Therapeutics, M.D. Anderson Cancer Center, Houston, TX

Solid tumors release soluble factors causing migration of a subset of normal bone marrow derived cells (BMDCs) – primarily VEGFR-1+ hemopoietic progenitor cells and VEGFR-2+ circulating endothelial progenitor cells – from the bone marrow to organ sites. These normal BMDCs self-assemble into a pre-metastatic niche - a micro-environment eventually hosting migratory tumor cells from the primary site. Disruption of this niche or selective targeting the migratory tumor cells may inhibit metastatic spread. The significance and specific characteristics of the BMDCs is still unclear. We have previously observed migration of VEGFR-1+ BMDCs to the lung and lymph nodes, and VEGFR-2+ BMDCs to liver, lung and lymph nodes. Using GFP+ BMDCs from genetically engineered mice, we characterized the role that recruitment of BMDCs may play in breast cancer metastasis. We assessed the BMDC recruitment profile in lethally irradiated female nude mice transplanted with GFP+ BMDCs from donor mice, followed by orthotopic placement of MDA-MB-231/Luc cells or injected with MDA-MB-231/Luc conditioned media for 30 days. Flow cytometry results show a gradual increase in the recruitment of CD11b+Gr1-1 cells in all the tissues examined from tumor-bearing mice. Recruitment of these cells to the liver in mice treated only with MDA-MB-231-Luc conditioned media was also apparent, but the increase was not as high as in mice of the orthotopically-placed tumors. Recruitment of CD11b+Gr1-1 BMDCs was also observed but only in liver and lung. Interestingly, conditioned media seemed to recruit this subset of cells more strongly to these two tissues than to organs from orthotopically placed tumors. Spleen and lymph node showed minimal recruitment of CD11b+/VEGFR-2+ BMDCs. We have previously developed the GrB/VEGF fusion construct, a novel pro-apoptotic fusion protein which specifically targets cells harboring VEGFR and have utilized this agent to specifically target BMDCs which are VEGFR+/- Our preliminary data indicate that treatment with GrB/VEGF does not significantly alter the recruitment of VEGFR-1+ and VEGFR-2+ cells to lungs, when assessed twice after the first week. We observed increased recruitment of F4/80+ macrophages to the lung. On the other hand, CD11b+Gr1-1 BMDCs were significantly reduced following GrB/VEGF treatment, although the F4/80+ cell population carrying this signature increased. Studies are ongoing to determine whether systemic administration of this agent can disrupt the formation of the niche and the eventual establishment of metastatic tumors. Understanding the role of BMDCs in metastatic spread and the formation of the pre-metastatic niche and their role in the early development of metastases will be critical in designing targeted therapeutic approaches to inhibit the metastatic process. Research sponsored, in part, by the Clayton Foundation for Research.

**GrB/VEGF121**
- 80 kDa homodimer (disulphide linked) fusion toxin composed of VEGF121 and the serine protease Granzyme B
- VEGF121 binds only to VEGFR1 (Flt –1/Flt1) and VEGFR2 (Flk –1/KDR)
- Mechanism of action of Granzyme B is well characterized but its impact at the genomic level is not known

**Granzyme B (GrB)**
- 25 kDa Human serine protease
- Normally enters cells through perforin-generated pores
- Causes release of cytotoxic c from mitochondria
- Initiates apoptosis by activating procaspases –3, -7 and -9
- Cleaves nuclear matrix proteins
- Can initiate apoptosis via caspase-independent mechanisms

**Expression and purification of GrB/VEGF121**
- Purification of Pro-GrB/VEGF121 on IMAC

**Flow cytometry gating strategy**
- Percent of GFP+ VEGFR-1+ BMDCs
- Day 35 Day 63 MDA-MB-231 CM
- 0 5 10 15 20
- Sp e e n L y m p h  N o d e s L iv e r L u n g
- Percent GFP+ VEGFR-1+ BMDCs
- Recruitment of CD11b+Gr1-1 subsets to lungs suppressed by GrB/VEGF121

**Summary**
- GrB/VEGF121 from genetically engineered mice were used to characterize the role of BMDCs in breast cancer metastases.
- Tumor placement resulted in increased migration of CD11b+Gr1-1 BMDCs to lungs and lymph nodes, and in reduced recruitment of BMDCs to liver, lung and lymph nodes.
- Injection of MDA-MB-231/Luc conditioned media significantly increased recruitment of BMDCs to liver and lungs.
- GrB/VEGF121 suppressed the recruitment of CD11b+Gr1-1 cells to lungs.
- Storks are engaging to demonstrate the targeted efficacy of BMDC signatures mediated tumor metastasis by GrB/VEGF121 and other agents.