The role of galectin-9 in metastatic melanoma and pregnancy, and its impact on outcome

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**Background**

- Pregnancy and cancer share many physiological processes including rapid growth, invasion into healthy tissue and immune escape through the induction of tolerance.
- Galectin-9 is a carbohydrate binding protein which interacts with TIM3 on activated T cells to shut down immune responses and maintain homeostasis.
- Galectin-9/TIM3 may be another immune checkpoint which plays a critical role in maintenance of pregnancy and progression in cancer.

**Objectives**

The aims of this research include:

- Determine the physiologic levels of soluble galectin-9 in pregnant women and metastatic melanoma patients compared to healthy controls
- Study the anatomy and location of galectin-9 and PD-L1 expression on tumors and placentas
- Correlate galectin-9 levels to survival in stage IV melanoma patients
- Characterize the in vitro effects of galectin-9 on peripheral blood mononuclear cells

**Methods**

- **Patient samples**: Study was approved by the institutional review board (T4-009025). Plasma samples were stored at -80 degrees before use. Tissues were requested from the pathology research core.
- **Galectin-9 concentration**: Soluble galectin-9 was measured using enzyme linked immunosorbant assays (ELISA).
- **Tissue staining**: FFPE healthy placentas and metastatic melanoma tumors were sectioned and immunohistochemistry (IHC) stained for PD-L1 and galectin-9 expression. Slides were visualized using IHCscore.
- **Survival curve**: Cutoff Finder® was utilized to determine the appropriate cutoff value between high and low galectin-9 patients. ROC found 1553 pg/mL resulted in a 82% sensitivity and specificity with the appropriate cutoff value between high and low galectin-9 levels to survival in stage IV melanoma patients. ROC curve showed patients with plasma galectin-9 levels higher than 1553 pg/mL have a worse overall survival (12.2 months) compared to those with galectin-9 levels lower than 1553 pg/mL (15.8 months; HR = 1.4557, p-value = 0.0476).
- **Mixed lymphocyte reaction**: Average and standard deviation of three independent autologous MLR using PBMCs isolated from healthy donors with or without exogenous galectin-9 (2ug/mL).
- **Flow cytometry**: PBMC subsets were measured after a 24 hour incubation with galectin-9 (2ug/mL) using a Guava easyCyte 8H7 flow cytometer. T helper cells (CD3+CD4+), T helper 1 cells (CD3+CD4+TIM3+), monocytes (CD14+), activated dendritic cells (CD11c+CD86+) and natural killer cells (CD16+CD56+) were measured.

**In vitro Immune Tolerance**

**Discussion**

- Like PD-1/PD-L1, TIM3/Galectin-9 shuts down anti-tumor responses in melanoma and promotes fetal tolerance in pregnancy.
  - Galectin-9 concentration is predictive of outcome in melanoma patients.
  - Thus far, TIM3 targeted therapies have shown little clinical benefit in cancer patients.
  - Galectin-9 may work independent of TIM3 to shut down the immune system.
  - Current studies are addressing this important question through knockdown of TIM3

**References**

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