ISOFORMS OF NEUROPILIN-2 REGULATE DISTINCT MACROPHAGE FUNCTIONS AND ARE ASSOCIATED WITH UNIQUE TUMOR-ASSOCIATED MACROPHAGES IN MURINE AND HUMAN BREAST CANCER

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Background Tumor-associated macrophages (TAMs) exert profound influence over breast cancer progression, promoting immunosuppression, angiogenesis, and metastasis.¹ Neuropilin-2 (NRP2), consisting of NRP2a and NRP2b isoforms, is a co-receptor for heparin-binding growth factors including VEGF-C and Class 3 Semaphorins. Selective upregulation in response to environmental stimuli and independent signaling pathways endow the NRP2 isoforms with unique functionality.^{2, 3} We have shown that the two isoforms of NRP2 endow opposing functionality to tumor cells due to distinct signaling pathways, with NRP2b promoting metastatic behavior.³ Although NRPs have been shown to regulate macrophage/TAM biology, the role of NRP2 isoforms in TAM functionality has yet to be evaluated.

Methods To assess the contribution of NRP2 isoforms to macrophage biology, conditional NRP2a and NRP2b knockout mice and stable shRNA knockdown of NRP2a or NRP2b in Raw264.7 macrophages were generated. Phagocytosis, lysosomal processing of phagocytosed cargo, cytokine production, and influence on tumor cell migration were assessed in vitro using NRP2 isoform knockdown macrophages. NRP2 isoform expression was evaluated on TAMs from murine 4T1 and EO771 mammary carcinoma models using spectral cytometry and single-cell qPCR. NRP2 isoforms and approximated immune composition were evaluated in paired primary tumors and distant metastasis using RNAseq in a cohort of 99 breast cancer patients. High-dimensional myeloid phenotyping was performed on malignant pleural effusions (MPEs) from breast cancer patients or effusions of benign origin using 33-color spectral cytometry and unbiased computational analysis.

Results NRP2 isoform expression was significantly increased in TAMs from murine tumors compared to macrophages from healthy mammary glands. NRP2 isoforms in human primary and metastatic breast cancer were strongly correlated with one another and positively correlated with increased TAMs. Distinct phenotypes of NRP2 isoform-expressing TAMs in were present in 4T1 and EO771 mouse breast cancers and within MPEs from breast cancer patients which were associated with high levels of activation and potential response to a hypoxic tumor niche. Genetic depletion of either NRP2 isoform resulted in dramatic reduction of LPS-induced IL-10 production, defects in phagosomal processing of apoptotic breast cancer cells, and increase in cancer cell migration following co-culture. By contrast, inhibition of IL-6 production was specific in NRP2b knockdown cells while phagocytic uptake of labeled particulates was inhibited only by NRP2a knockdown.

Conclusions These results demonstrate that NRP2 isoforms regulate both shared and distinct functionality in macrophages and that NRP2 isoform expression identifies unique TAM subsets in breast cancer.

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