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Role of Annexin A1 Secreted by Neutrophils in Melanoma Metastasis

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Abstract: Annexin A1 (AnxA1) is highly secreted by neutrophils and binds to formyl peptide receptors (FPRs) to trigger anti-inflammatory effects and efferocytosis. AnxA1 is also expressed in the tumor microenvironment, being mainly attributed to cancer cells. As recruited neutrophils are player cells at the tumor sites, the role of neutrophil-derived AnxA1 in lung melanoma metastasis was investigated here. Melanoma cells and neutrophils expressing AnxA1 were detected in biopsies from primary melanoma patients, which also presented higher levels of serum AnxA1 and augmented neutrophil–lymphocyte ratio (NLR) in the blood. Lung melanoma metastatic mice (C57BL/6; i.v. injected B16F10 cells) showed neutrophilia, elevated AnxA1 serum levels, and higher labeling for AnxA1 in neutrophils than in tumor cells at the lungs with metastasis. Peritoneal neutrophils collected from naïve mice were co-cultured with B16F10 cells or employed to obtain neutrophil-conditioned medium (NCM; 18 h incubation). B16F10 cells co-cultured with neutrophils or with NCM presented higher invasion, which was abolished if B16F10 cells were previously incubated with FPR antagonists or co-cultured with AnxA1 knockout ($AnxA1^{-/-}$) neutrophils. The depletion of peripheral neutrophils during lung melanoma metastasis development (anti-Gr1; i.p. every 48 h for 21 days) reduced the number of metastases and AnxA1 serum levels in mice. Our findings show that AnxA1 secreted by neutrophils favors melanoma metastasis evolution via FPR pathways, addressing AnxA1 as a potential biomarker for the detection or progression of melanoma.

Keywords: neutrophil-depleted mice; melanoma patients; FPR antagonists; B16F10 cells; neutrophil–lymphocyte ratio (NLR)

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1. Introduction

The tumor microenvironment consists of a heterogeneous population of cancer cells and a variety of other cells including the resident and infiltrating host cells [1]. Infiltrating immune cells in the microenvironment distinctly influences the tumor progression. While T-cell-mediated anti-tumor immune response correlates with favorable disease outcomes and is the basis of cancer immunotherapy [2–5], the myeloid cells act as antigen-presenting cells to promote anti-tumor T-cell responses at the initial phases of tumorigenesis; however, lately, they have been effectors of the tumor progression [6–8].

Herein, the reduction of neutrophils implied in the serum AnxA1 secretion decrease, which may impair melanoma cell invasion and, consequently, its dissemination.

Our findings added AnxA1 secreted by neutrophils in the blood or at metastasis sites as a new player of the melanoma cell invasion, pointing to AnxA1 as a pivotal mediator secreted by neutrophils acting on cancer cells. These data open a venue for investigations about the mechanisms of AnxA1 secreted by neutrophils, such as in the blood as in tumor metastasis and to propose AnxA1 blood levels or AnxA1^{+} neutrophils as a biomarker of early detection or melanoma progression.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/cells12030425/s1. Figure S1: Obtention and characterization of mouse peritoneal cells; Figure S2: Characterization of melanoma cells. Figure S3: Neutrophil profile after 24 h of culture with melanoma-conditioned medium (MCM). Figure S4: Evaluation of the circulating neutrophils (Ly6G+) and monocytes (F4/80) on the 21st day after melanoma cells injection. Figure S5: Uncropped gel. Reference [80] is part of the Supplementary Materials document.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics in Research Committee of the School of Pharmaceutical Sciences/University of São Paulo (CAAE 277951120.0000.0067) and A.C. Camargo Cancer Center (CAAE 277951120.4.3001.5432). The animal use was approved by the Institutional Animal Care and Use Committee (IACUC) at the School of Pharmaceutical (CEUA FCF/USP 583).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

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