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Snail-overexpressing Cancer Cells Promote M2-Like Polarization of Tumor-Associated Macrophages by Delivering MiR-21-Abundant Exosomes

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Abstract

Epithelial-mesenchymal transition (EMT) is a major event during cancer progression and metastasis; however, the definitive role of EMT in remodeling tumor microenvironments (TMEs) is unclear. Tumor-associated macrophages (TAMs) are a major type of host immune cells in TMEs, and they perform a wide range of functions to regulate tumor colonization and progression by regulating tumor invasiveness, local tumor immunity, and angiogenesis. TAMs are considered to have an M2-like, i.e., alternatively activated, phenotype; however, how these EMT-undergoing cancer cells promote M2 polarization of TAMs as a crucial tumor-host interplay during cancer progression is unclear. In this study, we investigated the mechanism of EMT-mediated TAM activation. Here, we demonstrate that the EMT transcriptional factor Snail directly activates the transcription of *MIR21* to produce miR-21-abundant tumor-derived exosomes (TEXs). The miR-21-containing exosomes were engulfed by $CD14^+$ human monocytes, suppressing the expression of M1 markers and increasing that of M2 markers. Knockdown of miR-21 in Snail-expressing human head and neck cancer cells attenuated the Snail-induced M2 polarization, angiogenesis, and tumor growth. In head and neck cancer samples, a high expression of miR-21 was correlated with a higher level of *SNAI1* and the M2 marker *MRC1*. This study elucidates the mechanism of EMT-mediated M2 polarization through delivery of the miR-21-abundant exosomes, which may serve as a candidate biomarker of tumor progression and provide a potential target for intercepting EMT-mediated TME remodeling.

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Introduction

Tumor microenvironments (TMEs) contain the cellular and non-cellular components that closely interact with tumor cells to influence tumor growth, progression and metastasis. A key element of the TME is the host immune system that is composed of the major effector cells of the innate/adaptive immune system [1]. Among these, tumor-associated macrophages (TAMs) are the most abundant innate immune cells in the TME, and they play crucial roles in tumor progression [2]. In physiological environments, macrophages can be categorized into classically activated (M1) macrophages, which produce pro-inflammatory cytokines to exert the host-defense function [3], and alternatively activated (M2) macrophages that secrete anti-inflammatory cytokines for tissue regeneration and

Abbreviations: ChIP, chromatin immunoprecipitation; EMT, epithelial-mesenchymal transition; FBS, fetal bovine serum; TAM, tumor-associated macrophages; DLS, dynamic light scattering; TEM, transmission electron microscopy; HNSCC, head and neck squamous cell carcinoma; PBMC, peripheral blood mononuclear cells; TEX, tumor-derived exosome; TME, tumor microenvironment.

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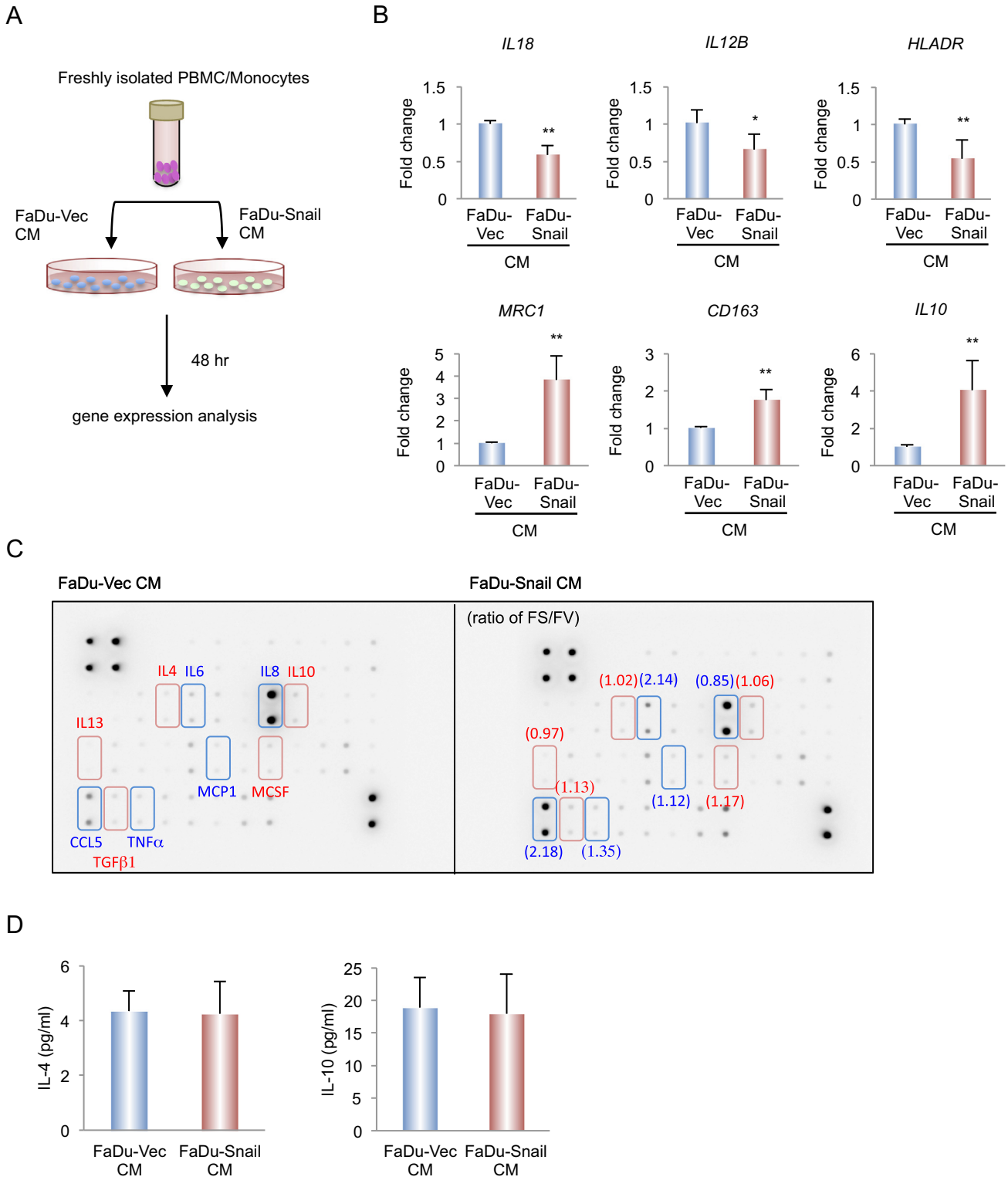

Figure 1. Snail-expressing cancer cells promotes M2-like polarization of macrophages. (A) Schema for representing the experiment procedure. (B) RT-qPCR for analyzing the expression of the markers of M1 (*IL18*, *IL12B*, *HLADR*) and M2 (*MRC1*, *CD163*, *IL10*) macrophages in $CD14^+$ human monocytes incubated with the conditioned medium from FaDu-Vec/FaDu-Snail for 48 hr. Data represents means ± S.D. * $P < .05$, ** $P < .005$ by Student's *t*-test. n = 3 independent experiments (each experiment contains 2 technical replicates). CM, conditional media. (C) Analysis of the conditioned media secreted by FaDu-Vec (left)/FaDu-Snail (right) by cytokine array. Blue, M1-promoting cytokines; red, M2-promoting cytokines. The ratio of FaDu-Snail/FaDu-Vec of the cytokines is shown in the right panel. CM, conditioned media. (D) ELISA for analyzing IL-4 and IL-10 from the conditional media of FaDu-Snail/FaDu-Vec cells. n = 3 independent experiments (each experiment contains 2 technical replicates). CM, conditioned media.