MSX1-Induced Neural Crest-Like Reprogramming Promotes Melanoma Progression

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Melanoma cells share many biological properties with neural crest stem cells. Here we show that the homeodomain transcription factor MSX1, which is significantly correlated with melanoma disease progression, reprograms melanocytes and melanoma cells toward a neural crest precursor-like state. MSX1-reprogrammed normal human melanocytes express the neural crest marker p75 and become multipotent. MSX1 induces a phenotypic switch in melanoma, which is characterized by an oncogenic transition from an E-cadherin−high nonmigratory state toward a ZEB1-high invasive state. ZEB1 up-regulation is responsible for the MSX1-induced migratory phenotype in melanoma cells. Depletion of MSX1 significantly inhibits melanoma metastasis in vivo. These results show that neural crest-like reprogramming achieved by a single factor is a critical process for melanoma progression.

Journal of Investigative Dermatology (2017) ■, ■−■; doi:10.1016/j.jid.2017.05.038

INTRODUCTION

The neural crest is a transient structure of the vertebrate embryo that gives rise to many types of derivative cells, including neurons and epidermal melanocytes. Neural crest cells migrate extensively to reach distinct sites within the developing embryo and those that migrate along the dorsolateral pathway give rise to melanocytes of the skin. The switch from a multipotent precursor toward a committed melanoblast is made early by expression of the melanocyte-restricted isoform of M-MITF, which is required both for fate determination and maintenance of melanocytic cells (Bentley et al., 1994; Hemesath et al., 1994; Yasumoto et al., 1994). We have recently shown that activation of Notch1 signaling alone is sufficient to reprogram foreskin-derived melanocytes to multipotent stem cells, which are functionally equivalent to neural crest cells (Zabierowski et al., 2011). Others have shown that ectopic endothelin-3 is able to convert clonal cultures of pigment cells to glial cells, putatively through a bipotent glial-melanocytic progenitor (Dupin et al., 2000). These studies imply that the stepwise differentiation from neural crest cells to melanocyte precursors, and then to mature melanocytes, is not a one-way road but can be reversed by reactivating genetic and/or epigenetic signatures that favor a stem cell-like phenotype.

MSX1 is a homeodomain transcription factor that plays an important role in the establishment of the early neural crest (Monsoro-Burq et al., 2005; Ramos and Robert, 2005). During early embryogenesis, a complex gene regulatory network coordinates the formation of the neural crest at the border of the neural plate and the non-neuralized ectoderm. Proteins expressed by the adjacent paraxial mesoderm determine the expression of transcription factors that act to induce neural crest formation; more specifically, intermediate concentrations of BMP4 induce the expression of MSX1 (Tribulo et al., 2003). The observation that ectopic expression of MSX1 is sufficient to dedifferentiate myotubes to mononucleated precursor cells (Odelberg et al., 2000) led us to hypothesize that the reactivation of MSX1 would dedifferentiate human melanocytes to a similar precursor state.

Malignant melanomas develop from melanocytes. Highly aggressive, therapy-refractory melanomas often lack pigment-related markers but instead express neural crest-specific genes (Bailey et al., 2012). The various implications of neural crest genes in cancer prompted us to

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Abbreviations: hESC, human embryonic stem cell; NCSC, neural crest stem cell; shRNA, short hairpin RNA

Received 3 March 2017; revised 30 April 2017; accepted 15 May 2017; accepted manuscript published online 16 September 2017; corrected proof published online XXX

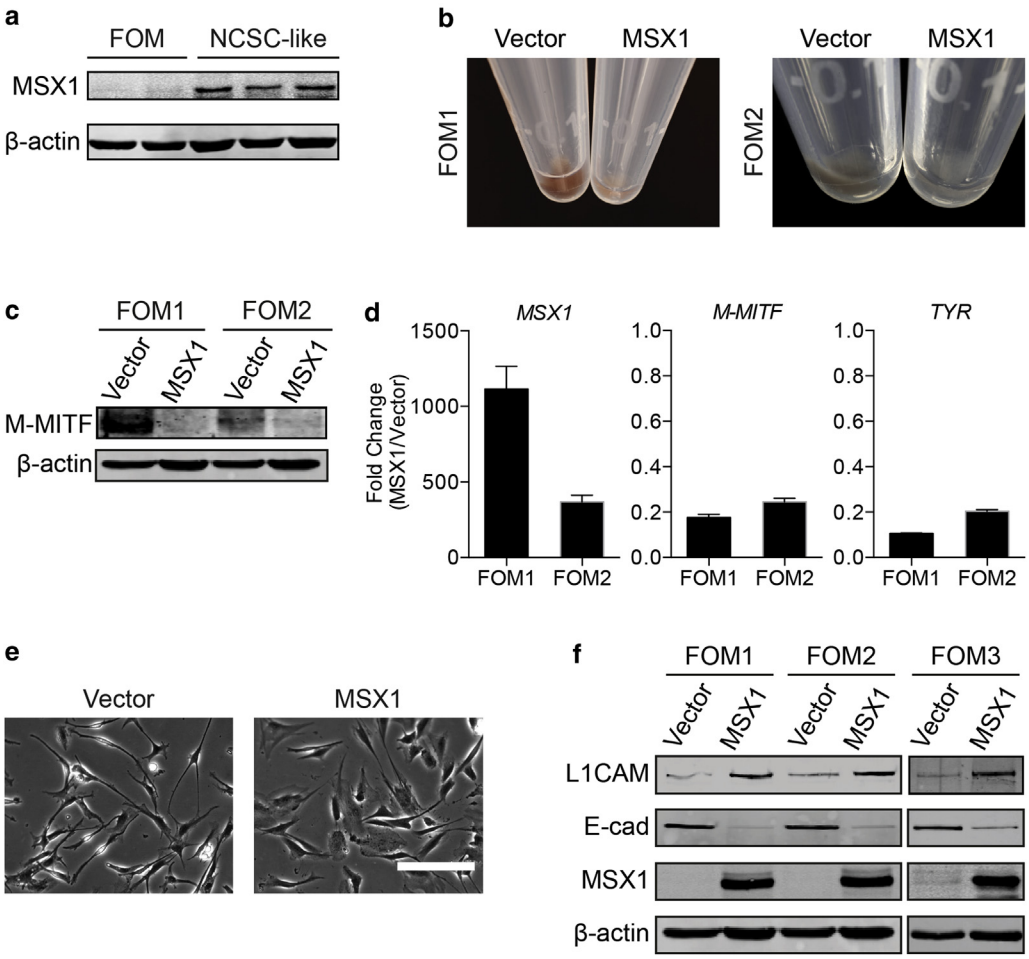

Figure 1. Expression of MSX1 attenuates pigmentation and the expression of melanocytic markers in melanocytes. (**a**) Immunoblot analysis of MSX1 in two foreskin-derived melanocytes (FOMs) and three foreskin-derived neural crest stem cell-like (NCSC-like) cells. (**b**) Macroscopic images of cell pellets from control vector and MSX1 vector-transduced melanocytes. (**c**) Immunoblot analysis of M-MITF in control vector and MSX1 vector-transduced foreskin-derived melanocytes. (**d**) Quantitative gene expression analysis of MSX1, MITF, and TYR in control vector and MSX1 vector-transduced melanocytes from two different donors. Error bars indicate one standard deviation of technical replicates. Sequences of the primers used are in Supplementary Table S1 online. (**e**) Phase images of control vector and MSX1 vector-transduced foreskin-derived melanocytes. (**f**) Immunoblot analysis of L1CAM, E-cad, and MSX1 in control vector and MSX1 vector-transduced melanocytes from three different donors. The left side images for FOM1 and FOM2 samples were derived from the same membrane used in **c**.

investigate whether MSX1 contributes to an aggressive phenotype in melanoma. Here, we examined the role of MSX1 in melanocytes and in melanoma. MSX1 is highly expressed in multipotent neural crest stem cell-like cells (NCSC-like cells) isolated from human dermis and in a panel of melanoma cell lines and patients' tissues. MSX1-transduced melanocytes lost pigmentation and gained the expression of neural crest markers, suggesting that these cells represent a dedifferentiated phenotype. Furthermore, these melanocytes were able to survive under human embryonic stem cell (hESC) culture conditions and were susceptible to differentiation into neuronal and mesenchymal lineages. Overexpression of MSX1 promoted cell motility in both melanocytes and melanoma cells and induced substantial changes in cell morphology, and silencing MSX1 by short hairpin RNA (shRNA) significantly inhibited melanoma migration in vitro and metastasis formation in vivo. Taken together, these results suggest that the neural crest-like reprogramming process conferred by MSX1 contributes to the metastatic spread of melanoma.

RESULTS

MSX1 attenuates pigmentation and alters the expression of adhesion molecules in melanocytes

Melanoma is considered to begin in transformed melanocytes. However, aggressive melanoma cells share many biological properties such as cell plasticity and invasion with the neural crest, which is the embryonic origin of melanocytes. To investigate the molecular mechanisms that dedifferentiate melanoma cells to a neural crest-like state, we used NCSC-like cells as a model. NCSC-like cells existing in human skin display the capacity for self-renewal and are able to differentiate into multiple neural crest-derived lineages, including melanocytes (Li et al., 2010; Toma et al., 2005). We have recently shown that differentiated melanocytes can be fully reprogrammed to multipotent NCSC-like cells by the reactivation of Notch1 signaling. These reprogrammed cells were devoid of pigment and lost expression of E-cadherin (Zabierowski et al., 2011). MSX1, which is an essential molecule for neural crest specification, was one of the most significantly up-regulated genes in Notch-induced NCSC-like cells (Tribulo et al., 2003; Zabierowski et al., 2011). Thus, we