

# Upregulation of myosin Va by Snail is involved in cancer cell migration and metastasis

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Cell migration, which involves acto-myosin dynamics, cell adhesion, membrane trafficking and signal transduction, is a prerequisite for cancer cell metastasis. Here, we report that an actin-dependent molecular motor, unconventional myosin Va, is involved in this process and implicated in cancer metastasis. The mRNA expression of myosin Va is increased in a number of highly metastatic cancer cell lines and metastatic colorectal cancer tissues. Suppressing the expression of myosin Va by lentivirus-based RNA interference in highly metastatic cancer cells impeded their migration and metastasis capabilities both *in vitro* and *in vivo*. In addition, the levels of myosin Va in cancer cell lines are positively correlated with the expression of Snail, a transcriptional repressor that triggers epithelial-mesenchymal transition. Repression or overexpression of Snail in cancer cells caused reduced or elevated levels of myosin Va, respectively. Furthermore, Snail can bind to an E-box of the myosin Va promoter and induce its activity, which indicates that Snail might act as a transcriptional activator. These data demonstrate an essential role of myosin Va in cancer cell migration and metastasis, and suggest a novel target for Snail in its regulation of cancer progression.

Cancer cell metastasis is a multistep, complex process including migration of detached cells from the primary tumor through the surrounding stroma, invasion of the cells into the circulatory system, extravasation and arresting at distant secondary sites. Many of these steps require cell motility, which is presumably driven by cycles of actin polymerization, cell adhesion and acto-myosin contraction.<sup>1,2</sup> In addition, metastatic cells have to undergo genetic and epigenetic changes to acquire their aggressive phenotypes, such as epithelial-mesenchymal transition (EMT).<sup>3</sup> Great progress has

been made in the identification of the molecular mechanisms underlying the whole metastatic process.

The zinc finger transcriptional factor Snail plays a central role in EMT as a transcriptional repressor through its direct binding to E-boxes in the promoters of a number of cancer metastasis-related genes such as E-cadherin, claudins, occludin and CYLD.<sup>4-7</sup> It also upregulates the expression of molecules involved in the degradation of basement membrane and extracellular matrix such as matrix metalloproteinases (MMP)-2 and 9 indirectly.<sup>8,9</sup> Furthermore, Snail acts downstream in EMT-inducing signal transduction pathways activated by TGF- $\beta$ , FGF, HGF and EGF growth factors, oncogenic Ras, integrin-linked kinase signaling, and hypoxia.<sup>10-12</sup> The expression of Snail has been observed to correlate with invasive and metastatic capabilities of various cancer cell lines and biopsies from cancer patients.<sup>7,13-15</sup> However, a direct link between Snail expression and proteins involved in cell motility apparatus has not been reported.

Myosins are a large family of structurally diverse actin-dependent molecular motors. All myosins utilize energy from ATP hydrolysis to generate force for unidirectional movement along actin filaments. The myosin superfamily consists of conventional myosins and several classes of unconventional myosins.<sup>16</sup> Unconventional myosin members play a variety of roles in organelle, RNA and protein transport, cell movement, signal transduction, cell morphology maintenance, and membrane trafficking.<sup>17</sup> However, their roles in cancer cell migration have drawn attention just recently as myosin VI was found to be involved in the dissemination of human ovarian cancer.<sup>18</sup> By using cDNA microarray analysis,

**Key words:** unconventional myosin, myosin Va, Snail, migration, metastasis

**Abbreviations:** CAM, chick embryo chorioallantoic membrane; EMSA, electrophoretic mobility shift assay; EMT, epithelial-mesenchymal transition; RT-PCR, reverse transcription-polymerase chain reaction; siRNA, small interfering RNA; shRNAi, short hairpin RNA interference.

**Grant sponsor:** National Natural Science Foundation; **Grant number:** 30671060; **Grant sponsor:** Program for New Century Excellent Talents in University of China; **Grant number:** NCET-07-0031

**DOI:** 10.1002/ijc.24641

**History:** Received 11 Dec 2008; Accepted 26 May 2009; Online 11 Jun 2009

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