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The ROCK signaling and breast cancer metastasis

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Abstract Metastasis is the predominant cause of death in most breast cancer patients. The molecular mechanisms underlying metastasis from primary tumors to distant organs are not clearly characterized. In this review, we depict the role of ROCK signaling in regulating cell motility and growth, and discuss the contribution of this signaling to breast cancer metastasis.

Keywords ROCK · Breast cancer · Metastasis

Abbreviations

AhR	Aryl hydrocarbon receptor
ER	Estrogen receptor
LIMK1/2	LIM kinase-1 and 2
miRNA	MicroRNA
MLC	Myosin light chain
MYPT1	Myosin-binding subunit
P-MLC	Phosphorylated MLC
PCBs	Polychlorinated biphenyls
ROCK	Rho-associated kinase
ROS	Reactive oxygen species

Introduction

Tumor progression from primary sites to distant organs (i.e. metastasis) is a hallmark for most malignant tumors. Once metastasis occurs, the disease essentially enters an incurable stage. To date, breast cancer is the most prevalent cancer among women and also the major cause of cancer death all over the world. Metastasis from primary tumors to other tissues accounts for more than 90% of breast cancer related mortalities. Breast cancer cells metastasize to specific distant organs with a ranked order of preference. Bone is the most frequent site of metastasis in breast cancer patients with a frequency of more than 80%, which is three times higher than lung or liver. Until now, the molecular mechanisms responsible for breast cancer metastasis remain to be elucidated.

The Rho family of small GTPases plays a critical role in regulating cell morphology, growth, apoptosis and motility. The Rho-associated kinases, of which there are two isoforms, ROCK 1 and 2 (here we refer to ROCK1 and 2 as ROCK), are principal mediators of Rho activity [1]. ROCK plays a crucial role in the regulation of in vitro invasion and motility and in vivo metastasis of breast cancer and other cancers [2]. In this review, we tend to delineate the role of ROCK signaling in breast cancer metastasis and interpret the molecular bases for the ROCK-induced pro-metastatic effects.

ROCK and its signaling

To detach and become motile is the first step in metastasis. The ability of cells to move requires actin meshwork-dependent adhesion and actin–myosin-driven contractility. The Rho-ROCK pathway plays a central role in these processes, and ROCK fundamentally controls the organization of actin cytoskeleton and cell movement by phosphorylating a number of downstream targets, such as myosin light chain (MLC). Phosphorylation of MLC by

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ROCK is the key event required for actin–myosin-based contractile force generation, which increases the assembly of filamentous myosin heavy chain and favors the binding between myosin and filamentous actin (F-actin) [3, 4]. Moreover, ROCK phosphorylates the myosin-binding subunit (MYPT1), and LIM kinase-1 and 2 (LIMK1/2) [2]. The increased phosphorylation of MYPT1 helps to prevent the dephosphorylation of MLC, and the phosphorylation of LIMK1/2 promotes their activity and subsequently leads to increased phosphorylation of cofilin proteins which can inhibit the latter's F-actin-severing capability [5]. Thus, the net effect of increased ROCK activity is to elevate force generation, and then facilitate cell adhesion, motility, and invasion. The role of ROCK in regulating actin–myosin contractility is described in Fig. 1.

In addition to targeting the cytoskeletal proteins, ROCK has also been demonstrated to target the oncogene, c-Myc [6–9], to thereafter influence the tumorigenic and metastatic features of cancer cells. c-Myc is one of a few transcription factors that are directly or indirectly controlled by ROCK [6–8]. Along with its partner protein Max, c-Myc regulates an estimated up to 15% of genes in the human genome and globally re-programs cells to drive proliferation [10, 11]. Aberrant regulation and overexpression of c-Myc are found in most tumor types and the c-Myc pathway is believed to play a critical role in oncogenesis [12–14]. Many studies have shown that c-Myc is a potential prognostic marker for recurrence and adverse outcomes in breast cancer patients [15, 16]. Amplification and overexpression of c-Myc is associated with distant metastases in human tumors including breast cancer

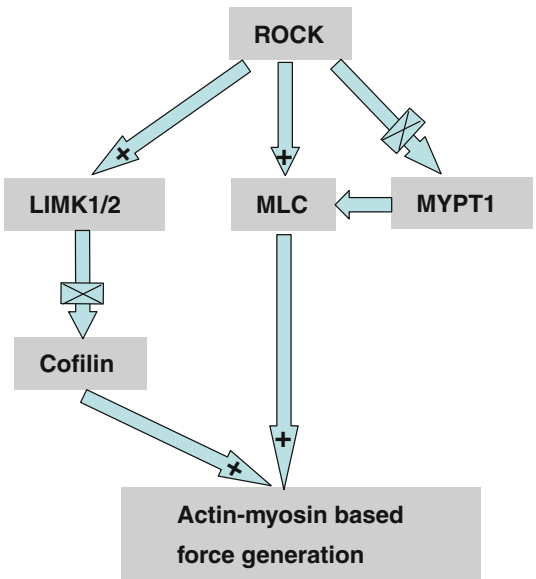

Fig. 1 The schematic about the role of ROCK in controlling the actin–myosin-based force generation

[13, 14, 17–19], and blockade of c-Myc using antisense molecules can inhibit metastasis [20].

The regulation of c-Myc by ROCK has been suggested by a few recent studies [6–8]. ROCK contributes to the stabilization of c-Myc protein via phosphorylation [8]. Our recent work discovered that the significant increase of c-Myc protein level in metastatic breast cancer cells in vitro and in vivo compared to non-metastatic cells corresponds to elevated ROCK protein level and its activity in these cells [9]. Importantly, inhibition of ROCK activity by either Y27632 (a specific ROCK inhibitor) or ROCK siRNAs could reduce the c-Myc protein level presumably due to the degradation of this protein resulting from diminished phosphorylation [9]. Furthermore, the miR-17-92 cluster was found to be involved in the ROCK signaling. In mammals, miRNAs are often transcribed as polycistronic primary microRNAs (miRNAs) that are then processed into several individual miRNAs [21], such as the miR-17-92 cluster. This cluster encodes 6 miRNAs in the human genome, i.e. miR-17, miR-18a, miR-19a, miR-20a, miR-19b and miR-92-1. Accumulating evidence supports that the miR-17-92 cluster is pro-tumorigenic and pro-metastatic. For example, it has been demonstrated to be overexpressed in various tumors, such as breast, prostate, lung, colon pancreas, stomach and lymphoma [22–24]. The transcription of this cluster is directly regulated by c-Myc. Our own microarray and real-time PCR analyses indicated that the expression of all miRNAs in this cluster is increased in metastatic breast cancer cells compared to non-metastatic breast cancer cells in vitro and in vivo, consistent with elevated ROCK activity in these metastatic cells [9]. Moreover, the expression of this cluster is significantly diminished upon ROCK inhibition with the ROCK inhibitor (Y27632) in all three breast cancer cell lines tested [9]. Additionally, blockade of endogenous miR-17 by anti-miR-17 molecules is shown to attenuate breast cancer cell invasion/migration in vitro and metastasis in a mouse model [9]. Together, the current studies support a positive regulation of ROCK on c-Myc and its downstream miRNAs, and the relationship between ROCK and c-Myc is worthy of further investigation.

The role of ROCK in breast cancer metastasis

Metastasis is usually recognized as a multi-step process through which tumor cells escape from primary sites, intravasate into vessels, circulate via blood stream, extravasate out of vessels and colonize distant organs, although the actual process may not be the case. Nonetheless, one of the prerequisites is tumor cells have to gain sufficient motility. So far, numerous studies have documented that Rho/ROCK pathway plays a central role in regulating cell motility.

Dysregulation of this pathway has been documented to be implicated in increased cell migration during tumor cell invasion and metastasis. Increased RhoA and RhoC expression was found in metastatic tumors, and the upregulation of RhoC was found to be a potential prognostic marker for tumors with boosted propensity to metastasize [25, 26]. RhoC is strongly induced in invasive ductal carcinomas, particularly those with distant metastasis [26]. Forced expression of wild-type RhoA conferred rat hepatoma MM1 cells enriched invasive ability both in vitro and in vivo [27]. Overexpression of RhoC enhances the in vitro invasion and in vivo metastasis in melanoma cells, while a dominant-negative RhoC reverses these capabilities [28].

As the principal downstream effectors of Rho, a large body of evidence has demonstrated that ROCK 1 and 2 are implicated in the regulation of in vitro invasion/motility and in vivo metastasis of breast cancer and other cancers [2]. A clinical study showed that the expression of ROCK1 is 10 times higher in human mammary tumors than normal control tissues [29]. And increased expression of ROCK1 correlates to higher pathological grade and metastasis, and its expression level is strongly linked to overall survival in breast cancer patients, as increased expression of ROCK1 corresponds to poor clinical outcome [29]. Our own study uncovered that the expression of ROCK 1 and 2, particularly ROCK1, is greatly increased in metastatic human breast cancer specimens compared to non-metastatic specimens, and is increased in late-stage tumors compared to early-stage tumors [9]. Overexpression of ROCK could significantly enhance the in vitro cell invasion/migration in breast cancer cells [30] and other types of tumor cells [31, 32]. Interestingly, we recently demonstrated that polychlorinated biphenyls (PCBs) profoundly enhance the in vitro motility and the in vivo metastasis of breast cancer cells by activating the ROCK signaling via induction of intracellular reactive oxygen species (ROS) independent of estrogen receptor (ER) and aryl hydrocarbon receptor (AhR) (unpublished data). Thus, elevated ROCK activity or ROCK signaling would promote the pro-metastatic capability of breast cancer cells. Conversely, the expression of the dominant-negative ROCK and the ROCK inhibitor, Y27632, could massively suppress in vitro cancer cell invasion/migration [31, 33–35] and in vivo motility and dissemination [31, 34]. We recently verified the significance of ROCK inhibition in breast cancer metastasis. The specific ROCK inhibitor (Y27632) or ROCK targeted-siRNAs reduce cell migration and proliferation in vitro and metastasis to bone in vivo using a novel "human breast cancer metastasis to human bone" mouse model [9]. ROCK inhibition diminishes not only the frequency but also the mass of metastases to bone with mild effect on primary tumor growth [9]. These data together suggest that augmented ROCK signaling contributes to breast cancer metastasis, and inhibition of ROCK or its induced signaling might stand for a potential therapeutics for metastases in mammary malignancies.

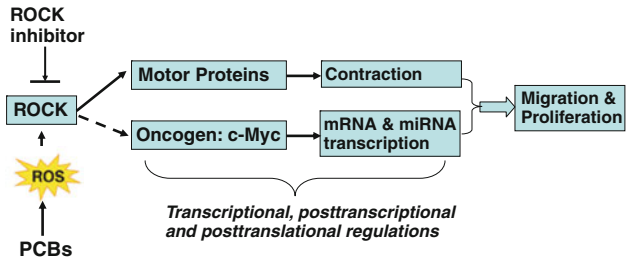

Fig. 2 The representation about the molecular mechanism responsible for the ROCK-stimulated signaling in promoting breast cancer growth and metastasis

Conclusions

The data discussed above collectively demonstrate that increased ROCK activity or its enhanced signaling stabilizes the actin cytoskeleton, enhances actin–myosin contractility and promotes the c-Myc pathway, including the transcription of c-Myc-regulated miRNAs. The combination of these processes likely augment cell invasion and migration, and increase the metastatic propensity of breast cancer cells.

To conclude, ROCK signaling is potently affecting primary tumor growth, invasive and metastatic features of breast cancer in a concerted series of events, at least in 3 ways (Fig. 2) (1) controlling the actin cytoskeleton and actin–myosin-dependent contractility; (2) targeting c-Myc to affect cell growth and survival; and (3) modulating the expression of miRNAs (the c-Myc regulated miR-17-92 cluster). Inhibition of ROCK-mediated signaling is a promising approach to suppress metastases in breast cancer, and this signaling presumably represents a novel target for treatment of breast cancer metastasis.

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Competing Interests None.

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