

Review

Overexpression of MICAL2, a novel tumor-promoting factor, accelerates tumor progression through regulating cell proliferation and EMT

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Received: 2017.08.13; Accepted: 2017.10.20; Published: 2018.01.01

Abstract

Molecule interacting with CasL 2 (MICAL2), a microtubule associated monooxygenase, is involved in cell growth, axon guidance, vesicle trafficking and apoptosis. Recent studies have demonstrated that MICAL2 is highly expressed in tumor and accelerates tumor progression and it is deemed to be a novel tumor-promoting factor. MICAL2 overexpression increases cell proliferation to accelerate tumor growth, and MICAL2 also promotes epithelial-mesenchymal transition (EMT)-related proteins to increase cancer cell metastasis. On mechanism, MICAL2 induces EMT by regulating SRF (serum response factor)/MRTF-A (myocardin related transcription factor A) signaling, Semaphorin/Plexin pathway and inducing ROS (Reactive oxygen species) production. In the present review, we introduced MICAL family, expatiated the structure and functions of MICALs, and summarized the mechanisms of MICAL2 involving tumor progression. The challenges and perspectives for MICAL2 in tumor are also discussed.

Key words: MICAL2, cancer, epithelial-mesenchymal transition, metastasis.

Introduction

MICAL (Molecule interacting with CasL) 2 is a member of MICAL family. MICALs are an evolutionary conserved family involved in cell growth, axon guidance, vesicle trafficking and apoptosis. MICAL was found by Suzuki *et al* in 2002 [1]. It was reported that there are one MICAL and MICAL-like (MICAL-L) protein in *Drosophila*, three MICALs (MICAL-1, MICAL-2, and MICAL-3) and two MICAL-L (MICAL-L1 and MICAL-L2) in *homo sapiens*, and eight MICALs including two paralogs for MICAL2, MICAL3, and MICAL-L2 in zebra fish [2]. MICALs are multidomain proteins containing N-terminal flavin monooxygenase (MO) domain, calponin homology (CH) domain, LIM domain, and C-terminal domain. But MICAL-like proteins lack of flavin monooxygenase domain. The functions of MICAL family are to participate in the control of cytoskeleton dynamics

and the related fundamental biological processes, such as cell-cell contacts, cell differentiation and migration, axon growth, dendrite pruning, formation of neuromuscular junctions, angiogenesis, vesicle trafficking and gene transcription [3].

In the recent, people further found MICALs' importance in the diseases, particular in tumors. MICAL2 prostate cancer variants (MICAL2-PV) overexpresses in prostate cancer cells with high Gleason score, and MICAL2-PV expression is correlated with c-Met expression [4]. c-Met can be activated by binding of semaphorin (Sema) 4D to plexin B1, and involve in metastasis and invasion of cancer cells [5]. Loria *et al* also demonstrated that MICAL1 and Sema6A are highly expressed in melanoma [6]. MICAL family regulates survival and migration of cancer cell by the Sema/plexin/mical pathway [5, 6]. In additional, MICAL1 overexpression

induces reactive oxygen species (ROS) generation and increases Akt phosphorylation in breast cancer cell [7]. ROS can induce the metastasis and invasion of cancer cells by epithelial-mesenchymal transition (EMT)[8-10]. MICAL-L2 protein is upregulated in ovarian cancer by Wnt/ β -catenin pathway, and the silencing of MICAL-L2 is correlated with the decrease of SNAIL, TWIST, ZEB1 and ZEB2 [11], which are the transcription factors of EMT [12]. MICAL2 knockdown results in ROS decrease, viability reduction, and loss of cancer motility and invasion [13]. In this review, we summarized the main functions of MICALs, and analyzed the mechanism of MICAL2 accelerating tumor, including SRF/MRTF-A pathway, Sema/Plexin pathway and ROS production.

The structures of MICALs

MICAL protein is composed of a flavoprotein monooxygenase (MO) domain, a calponin homology domain (CH), a LIM (Lin1-1, Isl-1 and Mec-3), and C-terminal domain (CTD) (Figure 1). The CTD includes a proline-rich region, a glutamic acid-repeat, and a coiled-coil motif [1]. However, MICAL2 doesn't contain CTD, and MICAL-like (MICAL-L) shares a same structure with MICALs except for N-terminal Redox enzymatic region (Figure 1). In human cells, MICAL-1 was mainly found in the cytosol, and MICAL-3 has also been found in the cytosol, while MICAL-2 was enriched in the nuclear [14].

The MO domain is located at the N-terminal part of MICAL protein, and covers about 500 amino acids. Within the MICAL MO domain, three separate conserved motifs can be discerned that defined the flavin adenine dinucleotide (FAD) binding domain (FBD) present in flavoprotein monooxygenases [3]. The main function of MO domain is NADPH-dependent methionine sulfoxidase activity. MICAL selectively modifies F-actin's methionine (M)

44 and 47 residues by adding two oxygens through MO domain [15]. This MO domain can produce ROS [16]. Collapsin response mediator proteins (CRMP) may serve a substrate binding to MO domain, and activate Sema1A axon guidance [17]. In the *Drosophila* test, MO domain can lead to a loss of axon repulsion induced by Sema [18]. And the monooxygenase inhibitor, epigallocatechin gallate (EGCG) can reduce the repulsive effects of Sema3A and Sema3F *in vitro* [19]. Zhou *et al* reported that MICAL1 can use its MO domain to suppress independently apoptosis [20].

The CH domain follows MO domain within MICALs. The main function of CH domain is as an actin-binding protein to involve in muscle contractility and non-muscle cell motility. There are three types of CH domain, type1, type2 and type3. The type1 and type2 domains are coordinated one by one in cytoskeletal proteins, and type2 can accelerate type1 to bind to F-actin [21]. The type 3 domain involves in regulating muscle contraction and signal proteins [22]. The recent studies showed that CH domain can cause an increase of K_{NADPH} , improve catalytic efficiency of monooxygenase reaction, but don't bind directly to F-actin [23]. CH domain advances recognition of F-actin and NADPH, and accelerates NADPH oxidation of MO domain activity in the presence of F-actin [24]. So CH domain is important to MO domain functions.

The LIM domain is composed of small cysteine-rich zinc-finger structures, and connected to CH domains by a variable region in all MICAL proteins. The zinc-finger of LIM domain serves mainly as a docking site for kinases, transcription factors, and cytoskeletal proteins, which involves in various cellular functions such as cytoarchitecture, cell adhesion and motility, and signal transduction [25]. For example, LIM domain of MICAL-L2 interacts with F-actin, and promotes F-actin cross linking [26].

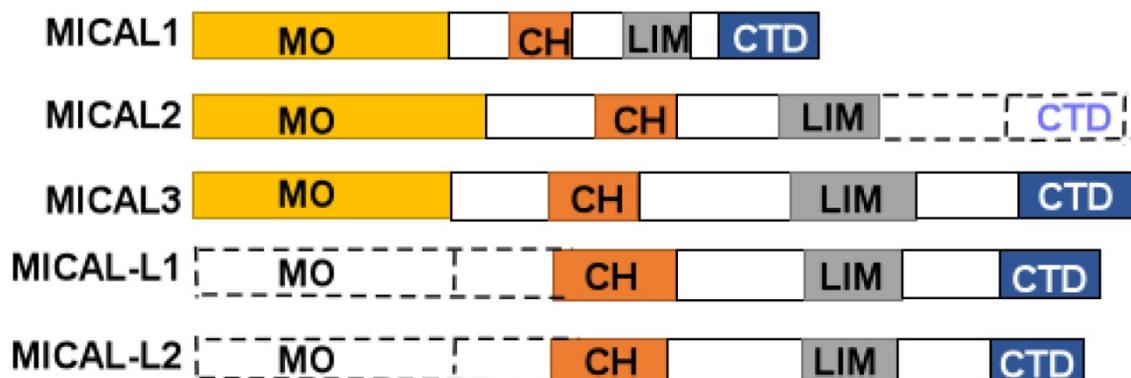


Figure 1. A diagram of MICALs structure. MICAL1 is composed of monooxygenase (MO), calponin homology (CH), LIM domain (LIM), and C-terminal domains (CTD). MICAL2 is composed of MO, CH, and LIM domains. MICAL3 is composed of MO, CH, LIM and CTD domains. MICAL-L1, 2 are composed of CH, LIM, and CTD domains.

The CTD domain is existed in C-terminal domain, and it is composed of a proline-rich region, glutamic acid-repeat, and coiled-coil motif. In the proline-rich region, there is a PPKPP (Pro-Pro-Lys-Pro-Pro) motif that interacts with SH3 domain of Cas family members, Cas, and CasL [1]. The glutamic acid-repeat region in MICAL-1 is poorly understood its function [19]. In the coiled-coil motif, there exists a plexin-interacting region that is similar to the alpha-helical region [18]. Furthermore, CTD domain may autoinhibit the activity of MO domain [17], likewise, CTD domain also interacts with vimentin, a protein components of intermediate filaments in mesenchymal tissues [1]. And CTD domain interacts with Rad small GTPases, regulating vesicle fusion at the plasma membrane [25].

MICAL family and its functions

MICALs have been found in brain, skeletal muscle, heart, fibroblasts, lung, kidney, bone marrow, thymus, spleen, liver, and testis – as well as in various cell lines and cancer cell lines [27]. MICAL proteins participate in various cellular functions. The main functions of MICALs in cell are redox regulation of cytoskeleton, regulation of axon guidance, dendritic pruning, and synaptic bouton distribution [3].

The important role of MICALs is as actin disassembly factors to directly regulate actin cytoskeleton and intracellular trafficking [28]. MICAL1 serves as a cytoskeletal regulator to connect CasL to intermediate filaments and interact with vimentin through COOH-terminal region, leading to cytoskeletal rearrangement [1]. In the *Drosophila* experiment, Hung *et al* demonstrated that MICALs alter the instability of F-actin and reorganize parallel F-actin networks into meshwork arrays [29]. All MICALs can mediate vesicle trafficking between endoplasmic reticulum and Golgi complex by binding Rab1A/B [3]. MICAL3 can link Rab8A and ELKS (a Rab6-interacting) to promote vesicle docking and fusion [25]. MICAL-L1 interacting with Rab8, Rab10, Rab13, Rab15, Rab35 or Rab36, can promote endocytic recycling of transferrin and β 1 integrin receptors through binding to EH domain-containing protein 1 (EHD1) on recycling endosomes and regulating trafficking [30]. The main function of EHD1 is interacting with Arf6 and Rabs to control membrane and receptor trafficking [31]. Likewise, Rab13 is mainly interacting with MICAL-L2 to mediate endocytic recycling of occluding [32]. In a recent work, Sun *et al* found that MICAL-L2 and Rab13 formed an insulin-dependent complex in vesicle traffic, which increasing Glucose transporter type (GLUT) 4 on plasma membrane [33].

NDR (nuclear Dbf2-related) kinase is a number

of the protein kinases that regulate cellular growth and apoptosis by catalyzing their relevant substrate. Mammalian STE20-like 1 (MST1) is a tumor suppressor protein regulating cell proliferation and apoptosis [34]. NDR1/2 kinase mediates apoptosis through MST1 downstream. MICAL1 can compete with MST1 for NDR binding to downregulate MST1-NDR apoptotic signaling pathway. MICAL1 combines with hydrophobic motif of NDR1 and colocalizes with NDR1 in cells, and ectopic expression of MICAL1 downregulates NDR kinase activation and activity [20]. Kanda *et al* reported that MICAL-L2 is involved in epithelial cell scattering. MICAL-L2 knockdown can inhibit Madin-Dar by canine kidney (MDCK) cells scattering, but re-expression of MICAL-L2 can rescue cells scattering. Rab13 and MICAL-L2 may decrease cell-cell adhesion and increase cell motility to break the balance between cell-cell adhesion and cell motility [35]. MICAL-L2 can bind to GTP-bound form of Rab13 by its C-terminus to mediate the endocytic recycling of occludin and the formation of functional tight junctions [32]. Rab13 is activated by its effector MICAL-L2 to recruit actin-binding proteins to promote migrating cell. Moreover, Rab13 can control the recycle of integrins and play a key role in cancer metastasis. Actinin-4 links MICAL-L2 to F-actin, and recruits MICAL-L2 to cell-cell junctions, and forms functional tight junction, which are associated with cell motility and cancer invasion [36]. Throughout epithelial junctional development from establishment to maturation of cell-cell adhesion, Rab13 and MICAL-L2 may regulate the reorganization of actin cytoskeleton [37]. Rab13 and MICAL-L2 regulate the transport of claudin-1 and occludin to plasma membrane. And Rab8 and MICAL-L2 also mediate the recycling of E-cadherin to plasma membrane by using a Ca^{2+} switch [38].

MICALs high expresses in tumors and promotes tumor progress

In *Drosophila*, *Mical* gene mutations were found, and gene mutation mediated abnormal MICALs high expression [39]. Xu Y, *et al* reported that *Mical* gene copy number was associated with MICAL expression, high gene number had high expression of MICAL2 [40]. In cellular experiments, low-serum condition induces MICAL2 overexpression [14]. MICALs expression is linked to various diseases including cancers [41]. MICAL2-PV overexpresses in prostate cancer cells, and MICAL2 may activate c-Met to increase invasive ability of prostate cancer cells [4]. Additionally, Sema6A and MICAL1 are highly expressed in melanoma, and MICAL1 as an effector of Sema/Plexin axis regulates Sema6A. Sema6A

complex with plexins regulates melanoma cell growth, motility, and invasion [6]. MICAL1 overexpresses in breast cancer cell, and promotes cell migration and invasion by activating ROS/Akt signaling [7]. Likewise, MICAL-L2 protein is up-regulated in ovarian cancer, and MICAL-L2 silencing suppresses ovarian cancer proliferation, migration and invasion ability [11]. It was also found that MICAL-L2 silencing induces ovarian cancer cells MET [11]. In a recent study, it is also found that MICAL2 overexpresses in gastric cancer (GC), non small cell lung cancer (NSCLC) and renal carcinoma, and MICAL2 mRNA expression is higher in GC diffuse histotype than intestinal histotype, is higher in NSCLC adenocarcinoma than squamous cell carcinoma. In additional, MICAL2 expression is high in aggressive primary cancer and metastatic emboli, but undetected in cancer cells at metastatic site [13].

MICAL2 participates in cell proliferation

In the renal carcinoma, *Mical2* gene expression has a relevance with differentiation and proliferation [13]. MICAL2 is involved in the proliferation of prostate cancer through mediating NADPH oxidation [42]. *Mical2* gene expression is correlated with the expression of *MKI67* in bladder cancer, and MICAL2 may involve in bladder cancer cell proliferation [43]. Ectopic expression of MICAL2 in breast cancer cell augments epidermal growth factor receptor (EGFR) expressions, further its mechanism is that MICAL2 triggers a delay in EGFR degradation. MICAL2 may promote cell proliferation and migration [44].

MICAL2 as a regulator of EMT in cancer metastasis

The gene expression analysis of cancer metastasis had proved MICAL2 as an important gene in EMT regulation [45]. EMT plays an important role in cell growth, tissue repair, stem cell transformation, and promoting migratory and invasive properties [12, 46]. EMT process is a loss of cell-cell adhesion and cell polarity, and acquisition of migratory and invasive properties. Furthermore, EMT induces stem cell properties, prevents apoptosis and senescence, and contributes to immune suppression [47, 48]. EMT process involves the loss of epithelial cell junctional proteins such as E-cadherin, claudins and ZO-1, and the increasing in mesenchymal markers such as N-cadherin, vimentin and fibronectin and cytoskeletal reorganization [49]. MICAL2 could oxygenate M44 and 47 amino acid residues on F-actin, resulting in F-actin disassembly and cytoskeleton dynamics [15, 42]. When F-actin depolymerization and cytoskeleton

dynamics change, the epithelial cell has ability of EMT.

1) MICAL2 regulates cell EMT through SRF/MRTF-A signaling pathway

Myocardin-related transcription factor (MRTF)-A, a cofactor of serum response factor (SRF) participates in EMT progress [50]. MRTF and SRF activities are dependent on Rho-actin signaling, Rho GTPases regulates G-actin and F-actin to influence the activation of SRF/MRTF-A-dependent gene transcription [51]. MICAL2 can induce F-actin depolymerization by Redox modification. F-actin depolymerization reduces G-actin, causing the interaction of SRF and MRTF-A, finally resulting in MRTF-A accumulation in nucleus (Figure 2). MRTF-A accumulation activates SRF/MRTF-A-dependent gene transcription [14]. And activation of SRF/MRTF-A-dependent gene transcription increases the capacity of adhesion, migration and invasion of cancer cells, and inhibits migration of adherent non-invasive cells [51, 52] (Figure 2). Additionally, MRTF-A is a downstream of transforming growth factor (TGF) β in the regulation of TGF β 1-induced EMT [53, 54]. MRTF-A could also stimulate transcriptional activation of actin cytoskeletal genes to change the organization of actin cytoskeleton, and downregulate E-cadherin and upregulate ZEB1, vimentin and slug expression in cancer cell [50, 55].

2) MICAL2 involves in cell EMT via Sema/Plexin pathway

In Sema/Plexin pathway, MICAL2 serves as a platform to recruit Sema to the site of plexin activation, which enhances MICAL2's monooxygenase activity or local intracellular concentration of ROS [56]. MICAL2 may also link Sema and plexin to depolymerize F-actin and induce actin rearrangement [29] (Figure 3). *Termam et al* reported that MICALs interact with neuronal plexin A receptor [18], so Sema, plexin, and MICALs constitute a Sema/plexin/mical pathway. The Sema/plexin/mical pathway involves in repulsive guidance, regulating cell morphology and migratory functions. Downregulation of E-cadherin and upregulation of β -catenin are the key step to EMT, Sema also affects the expressions of adhesion protein E-cadherin and β -catenin [57], and regulates tumor cell EMT [58]. MICAL2-PV overexpresses in prostate cancer cell, its expression is strongly concordant with classification of prostatitis, lymphatic metastasis, vascular invasion and Gleason scores of prostatic cancer. Likewise, it was found that MICAL2-PV expression is correlated with c-Met level, MICAL2 may upregulate c-Met to promote tumor cell invasive [4].

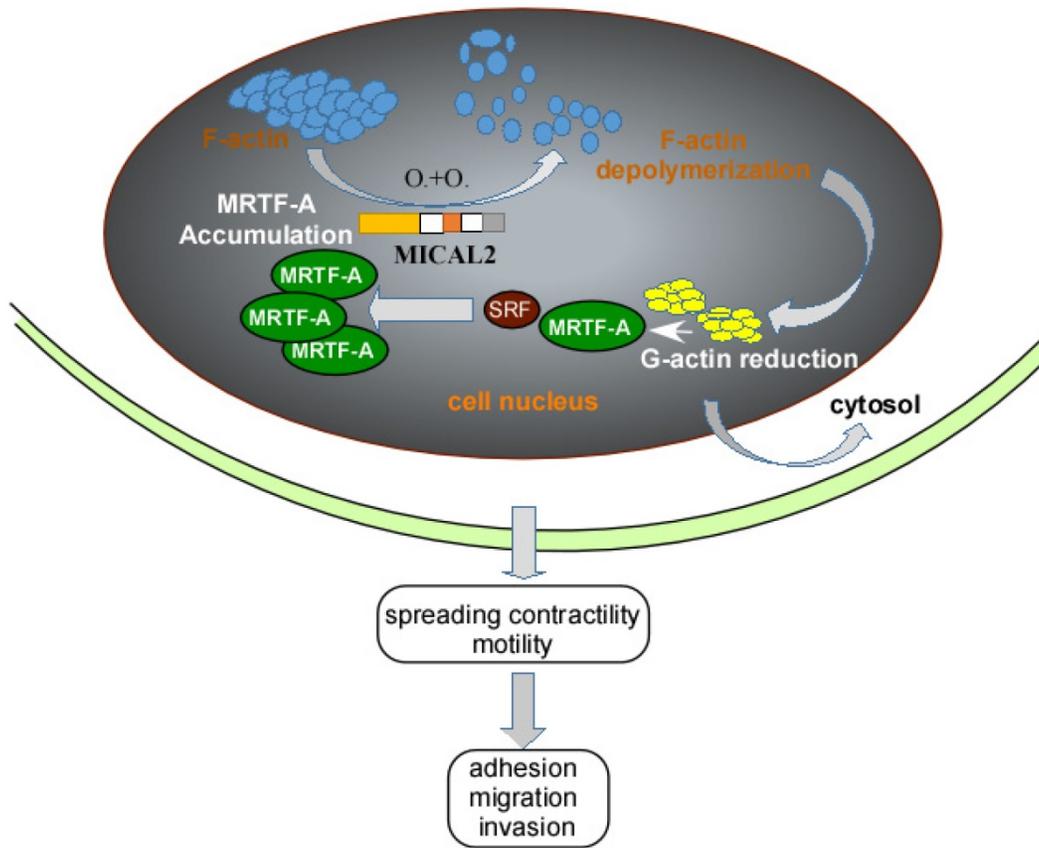


Figure 2. Schemastic illustration of MICAL2 functions. MICAL2 induces F-actin depolymerization by redox modification. The depolymerization of F-actin reduces G-actin and accumulates MRTF-A through clustering. The accumulation of MRTF-A in nucleus activates SRF/MRTF-A-dependent gene transcription, and finally promotes cancer cells migration and invasion.

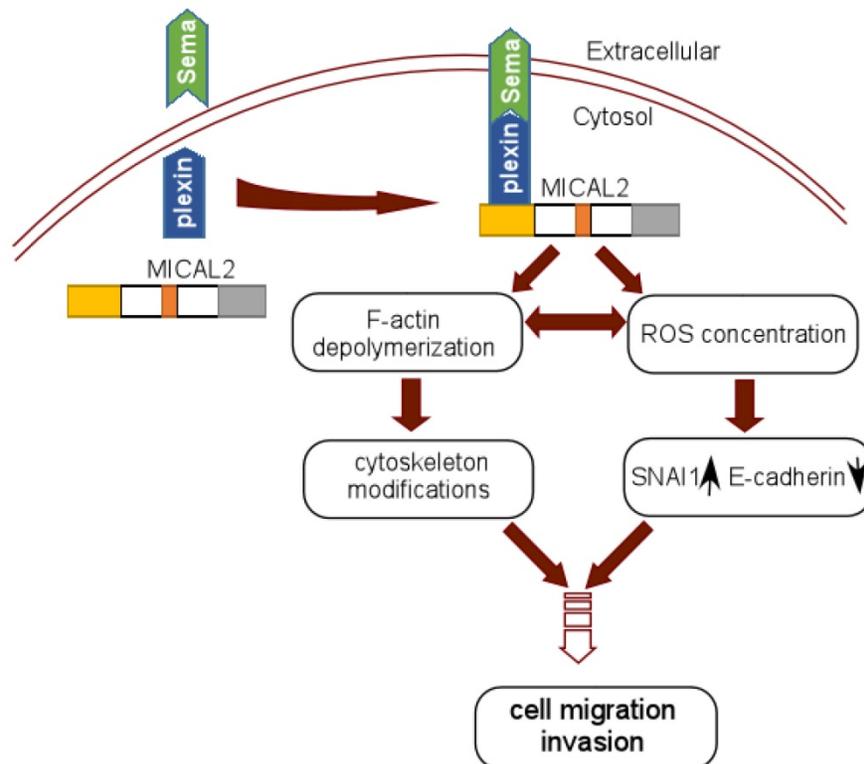


Figure 3. Schematic representation of MICAL2 as Sema platform. Sema interacts with plexin and activates plexin, and the activated plexin enhances MICAL2 monoxygenase activity and ROS intracellular concentration. MICAL2 accelerates F-actin depolymerization and increases cancer cell migration and invasion. ROS concentration induces EMT to promote migration and invasion.

Table 1. The functions of MICALs

MICAL	associated protein	Function	Reference
Mical-1	vimentin and CasL	cytoskeletal rearrangement	[1]
	CRMP-2	Axon guidance	[28]
	PlexA-1	Axon guidance	[17]
	NDR1	apoptosis	[20]
Mical-2	F-actin	cytoskeletal rearrangement	[14]
	Rab1	vesicle trafficking	[29]
Mical-3	Rab8A and ELKS	vesicle docking and fusion	[25]
	F-actin	cytoskeletal rearrangement	[14]
Mical-L1	Rab8,10, 13,15,35 and36	recycling endosomes and regulating trafficking	[30]
	EHD1	Focal adhesion and endocytic function	[31, 32]
Mical-L2	Rab13	neurite outgrowth and exocytosis	[33, 34]
	F-actin	cytoskeletal rearrangement	[35]

3) MICAL2 mediates cell EMT through inducing ROS production

MICAL2 induces constitutive F-actin depolymerization by increasing the generation of ROS [26]. Some experiments confirmed that ROS increase promotes EMT in cytoskeleton dynamics and microenvironment [10] (Figure 3). MICAL2 uses NADPH to produce ROS through its MO domain binding to FAD [13]. This process produces H₂O₂, and then H₂O₂ affects actin filaments, subsequently causes actin remodeling [16]. On the one hand, ROS generation mediated by MICAL2 is significant for actin-regulatory function. On the other hand, the high levels of ROS can increase metabolic activity, mitochondrial dysfunction and peroxisome activity, and deregulates cellular receptor signaling, oncogene activity and enhances activity of COXs, LOXs and thymidine phosphorylase [59]. ROS can promote EMT process by stimulating overexpression of RTKs, EGFR and c-Met/HGFR, and hyper activation of Rac-1 GTPase, hypoxia and Ras [10]. ROS also induces the repression of E-cadherin and the increase stability of SNAIL, and cell-cell disengagement [10].

Conclusions

In summary, MO domain is a critical structure of MICALs, and its pivotal function is redox regulation to the cytoskeleton. MICAL2 induces cancer cell invasion and proliferation, its mechanism is to induce F-actin depolymerization by redox modification. F-actin depolymerization interacts with MRTF-A and results in MRTF-A accumulation. MRTF-A accumulation in nucleus activates SRF/MRTF-A-dependent gene transcription, promoting cancer cells migration and invasion. In addition, MICAL2 also mediates ROS production, activates wnt/ β -catenin signaling and Sema/Plexin pathway, and promotes tumor cell EMT. However, some mechanisms of MICALs involving cancer progress are still undefined.

Acknowledgements

We thank the members in Clinical Laboratory of Zhuhai Hospital for contributions and helpful discussion.

Author contributions

Cai Y wrote the paper. Tang F and Lu J designed and revised the paper.

Funding

This work was supported in part by the National Natural Science Foundation of China (81372282, 81402368, 81402265, 81502346), Program for New Century Excellent Talents in University, NCET (NCET-06-0685), Guangdong Natural Science Foundation (S2013010013360), China Postdoctoral Science Foundation funded project (2016M592580).

Competing Interests

The authors have declared that no competing interest exists.

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