Overview of Ca\(^{2+}\) signaling in lung cancer progression and metastatic lung cancer with bone metastasis

Manh Tien Tran*  
Department of Dental Pharmacology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama 700-8525, Japan  
*Correspondence: Manh Tien Tran, Department of Pharmacology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama 700-8525, Japan. tran.tienmanh.1508@gmail.com  
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Abstract  
Intracellular Ca\(^{2+}\) ions that are thought to be one of the most important second messengers for cellular signaling, have a substantial diversity of roles in regulating a plethora of fundamental cellular physiology such as gene expression, cell division, cell motility and apoptosis. It has been suggestive of the Ca\(^{2+}\) signaling-dependent cellular processes to be tightly regulated by the numerous types of Ca\(^{2+}\) channels, pumps, exchangers and sensing receptors. Consequently, dysregulated Ca\(^{2+}\) homeostasis leads to a series of events connected to elevated malignant phenotypes including uncontrolled proliferation, migration, invasion and metastasis, all of which are frequently observed in advanced stage lung cancer cells. The incidence of bone metastasis in patients with advanced stage lung cancer is estimated in a range of 30\% to 40\%, bringing about a significant negative impact on both morbidity and survival. This review dissects and summarizes the important roles of Ca\(^{2+}\) signaling transduction in contributing to lung cancer progression, and address the question: if and how Ca\(^{2+}\) signaling might have been engaged in metastatic lung cancer with bone metastasis, thereby potentially providing the multifaceted and promising solutions for therapeutic intervention.

Keywords  
Lung cancer, Ca\(^{2+}\) signaling, bone metastasis, osteoclasts, bone microenvironment

Introduction  
Intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)\]) signaling is implicated in regulation of a variety of physiological processes deciding either cell survival or death. In unexcited states, (Ca\(^{2+}\)) ions are maintained at a very low level (in a range of 50-150 nM) nonetheless, a transient elevation of (Ca\(^{2+}\))\(_{i}\) obtained through either (Ca\(^{2+}\))\(_{i}\) efflux from intracellular organelles into cytosol or through (Ca\(^{2+}\))\(_{i}\) influx into cytosol from extracellular milieu, could mediate activation of various downstream signaling cascades [1]. Specifically, (Ca\(^{2+}\))\(_{i}\) fluctuation is tightly regulated by a series of Ca\(^{2+}\) channels, pumps and/or exchanges. Dysregulation of (Ca\(^{2+}\))\(_{i}\) homeostasis is a cause of the certain diseases such as developmental disorders, hypertension, cardiovascular disease, diabetes, Alzheimer's disease, and...
cancer [2, 3]. In the context of cancer, whether dysregulated (Ca\textsuperscript{2+}) homeostasis is necessary for malignant initiation has been disputable; however, there have been increasing cues proposing that dysregulation of (Ca\textsuperscript{2+}), homeostasis might be a central point of defects in mechanisms upon tumor promotion.

The Ca\textsuperscript{2+} channels, pumps and/or exchanges at plasma membrane (PM) predominantly mediate the intermittent Ca\textsuperscript{2+} flux from outside to inside of cells such as voltage-gated Ca\textsuperscript{2+} channels (VGCCs), specific receptor-operated channels (ROCs) and store-operated Ca\textsuperscript{2+} channels (SOCs), which are stimulated by membrane depolarization, by the external agonists and by depletion of internal Ca\textsuperscript{2+} stores, respectively. Meanwhile, the inositol-1,4,5-trisphosphate (IP\textsubscript{3}) receptor (IP\textsubscript{3}R) and the Ryanodine receptor (Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release channels-RyR) are two key Ca\textsuperscript{2+} receptors releasing Ca\textsuperscript{2+} from the internal stores such as endoplasmic reticulum (ER). Mechanistically, binding of IP\textsubscript{3} ligand to IP\textsubscript{3}R triggers IP\textsubscript{3}R activation, resulting in Ca\textsuperscript{2+} release from ER into cytosol [4] whereas RyRs, whose activity is dependent upon (Ca\textsuperscript{2+}), concentration, release (Ca\textsuperscript{2+}) from ER into cytosol in different cell types such as neurons, muscle cells, and epithelial cells [5, 6]. In addition, there are two leading systems responsible for Ca\textsuperscript{2+} extrusion across PM, including (1) the plasmalemmal Ca\textsuperscript{2+}-ATPase (PMCA), a calmodulin (CaM)-dependent Ca\textsuperscript{2+} ATPase regulating contractility in vascular, bladder and uterine smooth muscle [7], and (2) the electrochemically driven Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX), which is a bi-directional transporter exchanging three Na\textsuperscript{+} for one Ca\textsuperscript{2+} critically regulating (Ca\textsuperscript{2+}), in heart [8]. Besides, (Ca\textsuperscript{2+}), accumulation into ER could be mediated by the sarco-ER Ca\textsuperscript{2+}-ATPase (SERCA), ubiquitously present in ER of all eukaryotic cells. For instance, SERCA played a role in promoting relaxation via pumping (Ca\textsuperscript{2+}) into the lumen of sarcoplasmic reticulum (SR) that is a major subcellular pool of Ca\textsuperscript{2+} [9].

Lung cancer, also known as lung carcinoma, is the most frequently diagnosed malignancy and the leading cause of cancer death globally. Two major types of lung cancer best characterized include small cell lung carcinoma (SCLC) and non-SCLC (NSCLC), the latter accounting for approximately 85% of all lung cancers spreads locally to the thoracic cavity and to distant organs including bone [10]. Specifically, a range of 30%-40% of patients diagnosed with advanced stage lung cancer might have developed bone metastasis in a course of their etiological progression, bringing about a significant negative impact on both morbidity and survival [11]. Neoplastic bone formation is primarily derived from dysregulation of bone remodeling and homeostasis, tightly controlled by two functionally interrelated types of cells, (1) osteoblasts (OBs), which account for bone formation and (2) osteoclasts (OCs), which are responsible for bone resorption. It has been demonstrated that the “horrible consequence” of bone metastasis occurs as metastatic cancer cells enable to stimulate bone-resorbing activity of OCs, thereby leading to enhanced bone resorption [12]. Importantly, Ca\textsuperscript{2+} ions and cytokines released from osteoclast-triggered bone resorption promote tumorigenesis, contributing towards augmentation of tumor-propagating capacity of cancer cells and osteoclast-triggered bone resorption as well [13].

In summary, understanding of causes and consequences of regulatory mechanisms of (Ca\textsuperscript{2+}), signaling associated with lung cancer progression and development of metastatic lung cancer with bone metastasis may shed a light on the potential therapeutic targets or prognostic biomarkers for treatment of lung cancer patients with advanced stage lung cancer with bone metastasis.

**Dysregulated Ca\textsuperscript{2+} homeostasis and lung cancer progression**

As abovementioned, whether or not homeostatic disturbance of (Ca\textsuperscript{2+}), signaling, either transient or sustained, is one of major causes to initiate malignant events, comprising cell cycle, apoptosis, and metastasis, is still questionable. Nevertheless, followed by such malignant events, dysregulated (Ca\textsuperscript{2+}), signaling is frequently observed to contribute towards tumor progression. In this review article, the in-depth mechanisms upon contribution of dysregulated (Ca\textsuperscript{2+}), signaling towards lung cancer progression as well as metastatic lung cancer with bone metastasis were discussed.
The effects of dysregulated Ca\(^{2+}\) signaling on cell cycle

At early stage of tumor progression, cancer cells normally acquire a vast number of biological alterations that sustain their uncontrolled replicative capacity. Over last few decades, upon the development and technical modernization allowing to probe (Ca\(^{2+}\)), transient oscillation. The functional importance of (Ca\(^{2+}\)) signaling in regulation of cell cycle has been progressively unveiled. As a consequence of the greater than several thousand-fold gradient between (Ca\(^{2+}\)), and extracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) levels [14], the opening of cell surface Ca\(^{2+}\) channels leads to an immediate influx of (Ca\(^{2+}\)) across PM. Besides, the transient elevation of (Ca\(^{2+}\)), could be mediated through Ca\(^{2+}\) efflux from the internal Ca\(^{2+}\) stores such as ER, Golgi complex and the others.

Recently Ca\(^{2+}\) signals have emerged to be the hub of controlling G1 phase, G1/S and G2/M phase transitions [15]. In reality, cells are frequently sensitive to depletion of (Ca\(^{2+}\)), in G1, in which Ca\(^{2+}\) is critical for the expression of specific genes required for cell division such as FOS, JUN and MYC. Specifically, FOSL1, also known as aka FRA-1, a member of Fos family, is required for Kras-induced lung tumorigenesis in vivo, and promotes human lung adenocarcinoma proliferation and survival [16]. Besides, C-myc functions as a downstream signal of several growth factor receptors such as epidermal growth factor receptor (EGFR), transforming growth factor alpha (TGF\(\alpha\)), transforming growth factor beta (TGF\(\beta\)) receptor, interleukin (IL)-6 receptor, Notch receptor, and Frizzled receptor [17]. Importantly, C-myc also serves as one of the master transcription factors of many target genes that encode for proteins essential for regulation of cell growth and proliferation such as p15, p21, CDK4, CDC25A, E2F1 [18-22].

One of most important pathways regulated by (Ca\(^{2+}\)), signaling towards cell cycle progression is mitogen-activated protein kinase-renin-angiotensin system (MAPK-Ras) pathway. MAPK [rat sarcoma virus (Ras), rapidly accelerated fibrosarcoma (Raf) and mitogen-activated protein kinase (MEK)] pathway keeps a major role in regulating a variety of cellular processes such as proliferation, differentiation and survival. The abnormal expression of MAPKs is frequently observed in NSCLC [23]. It is best characterized that MAPK pathway is initiated by the external stimuli such as hormones, growth factors (GFs), cytokines and intracellular molecules, following the activation of the RAS upstream receptors including receptor tyrosine kinases (RTKs) and EGFRs. Activation of MAPK-Ras signaling pathway promoting cell cycle progression [24] by retinoblastoma (RB1) phosphorylation, which then triggers upregulation of cyclin D1-induced CDK4 or CDK6, eventually driving G1-to S-phase transition [25-32]. Deregulation of EGFR, also called ErbB-1, was found in a range of 40-89% of NSCLC [33]. Furthermore, (Ca\(^{2+}\)), is also crucial for regulation of several Ca\(^{2+}\)-dependent cascades such as calcineurin (CaN) and CaM-kinase. CaN, a Ca\(^{2+}\)- and camodulin-dependent serine/threonine protein phosphatase, plays a key role in promoting cell cycle progression at G1/S phase transition through cyclin D1 stabilization [34]. Liu et al. [35] identified that CaNA\(\alpha\), an isoform of CaN, which was overexpressed in lung cancer tissues, promoted cell proliferation through accelerating G1-to S-phase transition in SCLC cells in vitro. CaN inhibition by cyclosporin A (CsA) blocked the transcriptional activity of CREB binding protein (CBP) and the nuclear factor of activated T cells (NFAT), leading to alleviate the expression of pro-inflammatory cytokine genes [36, 37]. Noticeably, activation of transcription factors such as CREB and myocyte enhancer factor-2 (MEF-2) was regulated by (Ca\(^{2+}\)), elevation by the (Ca\(^{2+}\))\(_i\) influx across PM via L-type voltage-gated channels (LTCs) [38]. CsA-triggered CaN inhibition declined CDK2 activity by diminishing the expression of cyclin D1 during G1 [39], cyclin E and cyclin A [40]. Increases in (Ca\(^{2+}\)), concentration result in activation of CaN, which subsequently dephosphorylates NFAT proteins, allowing them to translocate to the nucleus to regulate the expression of the target genes [41].

Orai3 channels, frequently overexpressed in NSCLC, mediate Ca\(^{2+}\) entry via store-operated Ca\(^{2+}\) entry (SOCE) and promote cell cycle progression via Atk pathway [42]. Orai3 silencing downregulated MAPK kinase pathway via diminishing the phosphorylation form of ERK1/2, and expression of C-myc, which triggered cell cycle arrest in G1 phase in breast cancer [43]. On the contrary, overexpression of Ca\(^{2+}\) release-activated Ca\(^{2+}\) channel protein 1 (Orai1), also known as CRACM1, triggered reduction of store-operated Ca\(^{2+}\) influx and attenuation of EGF-mediated proliferative signaling and driving cell cycle arrest in A549 lung cancer cells [44]. Furthermore, antigen-stimulated opening of Ca\(^{2+}\) release activated Ca\(^{2+}\) (CRAC) channel, a highly Ca\(^{2+}\)-selective store-operated channel, enables the refilling of ER Ca\(^{2+}\) stores and maintain the persistency of Ca\(^{2+}\) oscillations
which are first identified as essential for T cell proliferation and cytokine production [45]. Conformational change and redistribution of stromal interaction molecule 1 (STIM1), the ER Ca\(^{2+}\) sensor, and Orai1, a key subunit of CRAC channel pore are required for activation of CRAC channel, which, in turn, triggers Ca\(^{2+}\) release from ER lumen into cytosol through activating IP\(_3\)R and/or (Ca\(^{2+}\))\(_e\) influx [46, 47]. Also, the depolarization-induced opening of VGCC Ca\(_{1.2}\) is directly suppressed by STIM1, causing a sustained internalization of VGCC Ca\(_{1.2}\) [48]. Heretofore, Wang et al. [49] identified that STIM1 was significantly overexpressed in lung cancer tissues as compared to that of non-neoplastic lung tissues; furthermore, Ge et al. [50] revealed that STIM1 knockdown induced cell cycle arrest at G2/M and S phases through alleviating expression of CDK1 and 2 in A549 and SK-MES-1 cells, and abolishing tumorigenesis and growth of lung cancer cells in nude mice xenograft. Upon reaching to cytosol, Ca\(^{2+}\) often forms complexes with the molecular components of “(Ca\(^{2+}\))\(_i\) signaling molecular toolkit” specific for each cell type given. Among such direct effectors essential for (Ca\(^{2+}\))\(_i\) signaling are CaM and Ca\(^{2+}\)/CaM-dependent protein kinases II (CaMKII), protein phosphatase 2B (PP2B) and protein kinase C (PKC), modulating the transcriptional activity of various transcription factors for a large number of genes required for cell cycle progression [51]. Using 1-[N, O-Bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine) (KN-62), a specific CaMKII antagonist, Williams et al. [52] observed that blockade of CaMKII activity inhibited the exponentially proliferative capacity of SCLC cells through ameliorating cell cycle arrest at S phase. 

| Ca\(^{2+}\)-channel/pump/exchanger and Ca\(^{2+}\)-handling proteins | Cell line | Expression | Described roles |
|---------------------------------------------------------------|-----------|------------|----------------|
| Orai3                                                         | NCI-H23 and NCI-H460 | ↑          | Decreased SOCE, abolished cell proliferation and triggered cell cycle arrest at G0/G1 phase [42] |
| Orai1/CRACM1                                                  | A549      | Not determined | Orai1/CRACM1 overexpression attenuated EGF-mediated store-operated (Ca\(^{2+}\))\(_i\) influx, and triggers G0/G1 cell cycle arrest [44] |
| STIM1                                                        | A549, SK-MES-1 | ↑          | STIM1 silencing inhibited colony formation, and induced cell cycle arrest at G2/M and S phases [50] |
| CaMKII                                                       | NCI-H69, NCI-H128, NCI-H146, NCI-H345 | Not determined | KN-62-induced inhibition of CaMKII activity triggered reduced DNA synthesis and cell cycle arrest at S phase [52] |
| CaNA\(_\alpha\)                                               | SBC-3     | ↑          | Promoted G1/S phase transition [35] |

↑: increased

**The effects of dysregulated Ca\(^{2+}\) signaling on apoptosis**

Apoptosis, a programmed cell death (PCD), is important for removal of mutated or transformed cells from the body essential for embryogenesis, development and tissue homeostasis of multicellular organisms. Principally, apoptosis comprises two core pathways: (1) the extrinsic pathway and (2) the intrinsic pathway, which are sequentially referred to as death receptor (DR)-mediated pathway and mitochondrial pathway [53]. Though stimulated in different manners, these pathways commonly converge into the same destination. Both the intrinsic and extrinsic apoptotic mechanisms lead to the activation of caspase 8, an initiator of a series of the apoptotic events through activating caspase 3, 6 and 7, subsequently resulting in cell collapse, chromatin condensation, breakdown of nuclear DNA, formation of apoptotic bodies and recognition of apoptotic cells by phagocytic cells [54].

During carcinogenesis, cancer cells acquired specific mechanisms of protection against apoptosis [55]. Among these, Ca\(^{2+}\) has been emerged as an important element exploiting its specialized effects towards regulation of apoptosis [56]. Under specific conditions at the initial step of mitochondria-induced apoptosis, overload of mitochondrial Ca\(^{2+}\), a vital sensitizer of the mitochondria permeability transition (MPT) triggers mitochondrial swelling, perturbation of the mitochondrial outer membrane [57]. MPT pore-triggered
release of the pro-apoptotic factors such as cytochrome c, apoptosis inducing factor (AIF), procaspase-9, Smac/DIABLO, and endonuclease G into cytosol causes a massive activation of proteases (caspases) and phospholipases [58-61]. To resist to apoptosis, cancer cells typically acquire the highly protective mechanisms against abolishment of mitochondria-triggered Ca\textsuperscript{2+} signals.

ER-derived Ca\textsuperscript{2+} signals, critical for regulating the apoptosis-related events, are also engaged in the mitochondria-induced apoptosis via the mitochondria-associated membranes (MAMs) juxtaposed between ER and mitochondria [62, 63]. Ca\textsuperscript{2+} storage in the ER is accomplished by the action of SERCA and of the intraluminal ER Ca\textsuperscript{2+}-binding proteins such as BiP, calreticulin and calnexin whereas the release of Ca\textsuperscript{2+} from the ER is virtually mediated by IP\textsubscript{3}Rs. The role of MAMs for regulation of (Ca\textsuperscript{2+}), homeostasis is mediated by IP\textsubscript{3}R3, RyR and SERCA [63]. Upon ER-derived Ca\textsuperscript{2+} signal-induced mitochondrial remodeling, B-cell lymphoma-2 (Bcl-2) proteins the first anti-apoptotic proteins identified regulate apoptosis via regulating Ca\textsuperscript{2+} transfer between ER and mitochondria [64]. These proteins are functionally categorized into the anti-apoptotic group (Bcl-2, BCL-X\textsubscript{L}, and Mcl-1) and the pro-apoptotic group (Bax, Bak, Bim, Bid, etc.). Anti-apoptotic Bcl-2 proteins regulates apoptosis by modulating the ER-mitochondrial Ca\textsuperscript{2+} transfer via the MAMs [65] while overexpression of pro-apoptotic Bcl-2 decreased both ER-Ca\textsuperscript{2+} release either by the direct control of IP\textsubscript{3}R3-mediated pore opening or by lowering the Ca\textsuperscript{2+} content of the ER, which weakens Ca\textsuperscript{2+}-triggered MPT, and thus enables cancer cell to resist to apoptosis [66]. Abnormal upregulation of pro-apoptotic Bcl-2 is frequently observed in various types of cancer cells such as gastric, colon, breast and lung cancer [67-70]. Indeed, alleviation of ER-Ca\textsuperscript{2+} levels and signals has been observed in pro-apoptotic Bax and Bak-knockout murine embryonic fibroblasts (MEFs) [71]. Strikingly, enhanced ER Ca\textsuperscript{2+} levels by ectopic expression of SERCA2 rescue their sensitivity to death stimuli, suggesting the functional necessity of Bcl-2 proteins in regulating the ER-mitochondrial Ca\textsuperscript{2+} gateway and cell death in MEFs [71]. Bcl-2 mutant has also been reported to reduce ER Ca\textsuperscript{2+} by inhibition of SERCA2 as a consequence of a reduction of SOCE [72, 73]. Bergner et al. [74] reported that a reduction of Ca\textsuperscript{2+} content correlated with a decreased expression of SERCA2 pumping Ca\textsuperscript{2+} into the ER, an increased expression of IP\textsubscript{3}R releasing Ca\textsuperscript{2+} from the ER in various types of lung cancer cell lines. In contrast, the detailed mechanisms underlying Bcl-2-mediated regulation of SOCE remains controversial. Depletion of Ca\textsuperscript{2+} in the ER causes translocation of the SOC channel activator, STIM1, to the PM [75]. Thereafter, binding of STIM1 to Orai1 and/or transient receptor potential channel 1 (TRPC1) forces them to open for allowing Ca\textsuperscript{2+} entry across the PM [75].

Oncogenic K-RAS, which degenerates ER Ca\textsuperscript{2+} dynamics [76], and Akt, which not only phosphorylates and inactivates several pro-apoptotic Bcl-2 such as Bad, Bax and hexokinase-2, but more importantly diminishes mitochondrial Ca\textsuperscript{2+} overload via allosterizing IP\textsubscript{3}, R opening, also contribute towards inhibition of the intrinsic apoptosis inhibition [77, 78]. Concomitantly, downregulation of protein phosphatase and tensin homolog (PTEN) in NSCLC tumors [79] antagonized F-box and leucine rich repeat protein 2 (FBXL2)-induced ubiquitination of IP\textsubscript{3}R, thereby stabilizing IP\textsubscript{3}R in ER [80]. Besides, EGFRs with its aberrant expression and constitutive activation in in NSCLC [81] stimulate three of the most well-characterized signaling branches such as Ras-MAPK, phosphoinositol 3 kinase (PI3K)-protein kinase B (PKB)/Akt and phospho lipase C (PLC)-PKC pathways, enhancing ER-mitochondria Ca\textsuperscript{2+} transfer, thereby abolishing mitochondria-induced apoptosis. Also, loss of promyelocytic leukemia protein (PML) isoform IV, a suppressor of transcriptional activity of EGFR, for instance, on cyclin D1 gene promoter in lung cancer cells [82] also contributed to the EGFR-mediated mitochondria-induced apoptosis and cell cycle arrest [81].

The effects of dysregulated Ca\textsuperscript{2+} signaling on metastatic lung cancer

Metastasis, a term used to describe the spread of cancer cells from the primary tumor to surrounding tissues and to distant organs, is the major cause of morbidity and mortality in cancer patients. Among all solid tumors, SCLC is one of the most aggressive malignancy associated with a majority of patients diagnosed with metastatic disease [83]. Metastatic SCLC cells easily dissociate from lungs to disseminate throughout bloodstream and/or lymph system to anatomically distant organs such as lymph nodes, brain, liver and bone [83].
Progress of metastatic cascades primarily begins with the loss of cell-extracellular microenvironment [extracellular matrix (ECM)] as well as cell-cell attachment. Cells are connected to the ECM at focal adhesion points by structural complexes linking membrane spanning integrins to the cytoskeleton. Therefore, migratory capacity of cancer cells are principally assessed by the rate of focal adhesion assembly and disassembly. Noticeably, calcium pulses promote the association of focal adhesion kinase (FAK), which regulates focal adhesion turnover, with the focal adhesion complex (FAC). More detailed, calcium pulses strengthen the FAK at the specific sites where it is phosphorylated in a CaMKII-dependent manner. The movement of migrating cells is initialized by the extension of the protrusive front edge, which is known as lamellipodia. Establishment of a gradient difference of (Ca^{2+}) levels, which was lower in the front, and higher in the rear of the migrating, polarized cells, caused rear retraction, focal adhesion (at the rear) and protrusion (at the front) [85, 86]. Following protrusion, the cell front starts to retract and locally adhere to ECM in lamella [87], which plays a pivotal in actomyosin contractility and F-actin disassembly in a treadmill-like manner [88].

Furthermore, actin and myosin, two of the important structural constituents, are regulated indirectly by calcium signaling via the activation of the cyclic element-mediated Ca^{2+}-dependent kinases, named calpain [89], and regulation of Rac1, RhoA, Cdc42, protein kinase A (PKA) [90, 91], and local Ca^{2+} signals between lamellipodia and lamella [92]. For retraction of the rear edge, calcium signaling play a vital role in maintaining contractivity and stabilizing the directional movement via modulating the Ca^{2+} influx through L-type Ca^{2+} channels [93].

Calcium-dependent MLC kinase (MLCK)-mediated phosphorylation of myosin light-chain (MLC) triggers myosin II-induced actomyosin contractility [94], promoting the retraction and adhesion more efficiently [95, 96].

It is well-characterized that “local Ca^{2+} pulses” in the front of migrating cells are released from ER via IP_{3}-induced activation of IP_3Rs [97], which is generated through activation of RTK-PLC-dependent signaling pathways. It is therefore proposed that ER-derived Ca^{2+} release by the axis of RTK-PLC-IP_3R would be major source of Ca^{2+} pulses in the front of migrating cells. Indeed, EGFR, also known as HER1, belonging to the ErbB family of structurally related RTKs that comprises four isoforms: ErbB2 (HER2), ErbB3 (HER3) and ErbB4/HER4 [98], is overexpressed and constitutively activated in 62% of NSCLC cases [99]. It is clear that EGFR is the key activator of the ERK/MAPK, Akt-Pi3K, and PLCγ-PKC signaling cascades [100]; furthermore, Tsai et al. [101] reported RTK and PLC were enriched at the leading edge of migrating cells, in correlation with intensity of local Ca^{2+} pulses in the cell front.

In addition to RTK, G-protein coupled receptors (GPCRs) on local Ca^{2+} pulses in the cell front via activating PLC, which hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) to release IP_3, which binds to IP_3Rs to trigger transient Ca^{2+} release [102, 103]. In a meanwhile, depletion of ER luminal Ca^{2+} sensitizes SOC channels located at PM to influx (Ca^{2+}) across PM [104]. Chantôme et al. [105] once reported that the interaction of Orai1 with SK3 channel, a potassium channel belonging to the small conductance Ca^{2+}-activated potassium (KCa) channel family, regulated the constitutive (Ca^{2+}) entry through Orai1 localization within the lipid raft, which affected the migratory ability of breast cancer cells. Disruption of interaction between SK3 and Orai1 from lipid rafts weakened SK3-mediated Ca^{2+} entry, migration and bone metastasis [105]. Moreover, STIM/Orai-mediated SOCE is also essential for elevation of (Ca^{2+}) level. STIM1, the ER Ca^{2+} sensor, and Orai3, constitute a native SOC entry essential for NSCLC progression [42, 50]. Increasing evidence implied that STIM1 assisted the turnover of cell matrix adhesion complexes, thereby enhancing cell migration by maintaining local Ca^{2+} pulses in the front of migrating cells [106]. In migrating cells, local Ca^{2+} pulses near its leading edge cause depletion of Ca^{2+} in the front ER, resulting in activation of STIM1 at the cell front [101]. More specifically, STIM1 was remarkably translocated to the ER-PM junction in cell front rather than cell rear during cell migration, thereby promoting maintenance of cell polarity and motility [101].

In addition, inhibition of the activity of Ca^{2+} permeable channels, PMCA and NCX, by the specific blockers, which are vanadate (V^{5+}) and KB-R7943, respectively, led to a decrease in migratory capacity of MDCK-F cells, suggesting these channels were of significant importance for cell migration [107]. Furthermore, inactivation of ER membrane-located SERCA, which is responsible for pumping (Ca^{2+}) into the ER lumen, triggering a leak of the ER luminal Ca^{2+} into cytosol [101]. The high (Ca^{2+}) concentration caused MLCK saturation and myosin...
contractility [101]. Indeed, Atousa Arbabian [108] once reported that dysfunctional SERCA diminishes the ER luminal Ca\textsuperscript{2+}, thereby disabling further Ca\textsuperscript{2+} signaling through IP\textsubscript{3}Rs, suggesting a physiological importance of SERCA on lung cancer progression, invasion and metastasis.

**Metastatic lung cancer with bone metastasis**

Bone is one of the most common metastatic sites for lung cancer, in which 36% of patients with bone lesions, and a range of 20%-60% with bone marrow micrometastasis [109, 110]. Metastasis lung cancer with bone metastasis is a major source of morbidity and mortality; however, it is not frequently detected in the patients until pain, skeletal-related events (SREs) in spine, ribs, pelvis and proximal long bones, pathological fractures and nerve compression syndromes occur [111]. Therefore, comprehension of why and how the various specific features of bone microenvironment, associated with spatio-temporal fluctuations of Ca\textsuperscript{2+} signaling network preferentially towards bone metastasis is essential for development of the efficacious drug program.

Bone is a dynamic organ included a variety of embryo-derived cells such as hematopoietic, stromal, endothelial, adipocytes, OCs, OBs and osteocytes [112]. Two important mediators of the hematopoietic stem cell (HSC) environment are (1) the chemo-attractant stromal derived factor-1 (SDF-1) or C-X-C motif chemokine ligand 12 (CXCL12) and (2) the cell adhesion factor (Annexin2 or ANXA2) [113]. CXCL12 regulates HSC homing to the bone marrow, while ANXA2 is likely involved in tumor binding to the osteoblastic niche, and may act as an anchor of CXCL12 and aid in localization to the niche [113]. The disseminated tumor cells (DTCs) could survive in a quiescent state in bone marrow of cancer patients for years. Increasing evidence suggests that DTCs gain access to the bone marrow using homing mechanisms similar to those of HSCs. The interaction of CXCL12, which is secreted by bone marrow stromal cells including fibroblasts and endothelial cells, to C-X-C motif chemokine receptor 4 (CXCR4), which is aberrantly expressed in tumor cells, allows tumor cells to directionally migrate to bone [114] mainly through upregulating the two most crucial downstream pathways comprising IP\textsubscript{3}K and MAPK pathways. Nonetheless, the detailed mechanisms of how CXCR4/CXCL12 interaction stimulates metastasis and/or tumor growth and their complete implications on metastatic lung cancer in bone are unknown.

Bone homeostasis is maintained by two major types of bone cells, consisting of OBs and OCs, which are responsible for bone formation and bone resorption, respectively [115]. Of these, OBs, differentiated from mesenchymal stem cells, participate in regulating bone remodeling by generating ECM and calcium phosphate crystals, which are deposited into the interstitial space of the matrix [116]. OCs, the polarized, multinucleated myeloid lineage cells, adhere to the bone surface through αvβ3 integrin, form ruffled borders, and secrete acid to solubilize calcium phosphate crystals as well as secret the collagenases and proteinases such as tartrate-resistant acid phosphatase (TRAP), matrix metalloproteinase 9 (MMP9), and cathepsin K (CTSK) that demineralize and degrade extracellular proteins such as type I collagen [117].

Bone has several particular characteristics such as acidified milieu, hypoxia (O\textsubscript{2} deficiency) and high level of (Ca\textsuperscript{2+})\textsubscript{2} enabling tumor cells to establish an acidic microenvironment via production of a large amount of lactic acid, which then creates the local areas inside bone, thereby accelerating tumor cell dormancy and promotes osteolysis [118]. The release of bone resorption-derived (Ca\textsuperscript{2+})\textsubscript{2} triggers activation of Ca\textsuperscript{2+}-sensing receptor (CaR), a G protein-coupled receptor, on PM of tumor cells and OBs [119], OCs [120] and especially tumor cells [121], including lung adenocarcinoma [122]. Activation of Ca\textsuperscript{2+}-sensing receptors enhances the secretion of parathyroid hormone-related peptide (PTHrP), which subsequently binds to its receptor, PTHR1, to increase receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL) expression in bone marrow stromal cells, thereby promoting osteolysis [121]. Furthermore, RANKL-mediated osteoclast differentiation triggers IP\textsubscript{3}R-induced local Ca\textsuperscript{2+} release, inducing activation of one of the master transcription factors of osteoclastogenesis, the NFATc-1 [123], subsequently entering nuclei to bind to the promoters of specific genes required for osteoclast differentiation [123]. In addition, it is unknown whether bone resorption-derived (Ca\textsuperscript{2+})\textsubscript{2} might have been responsible for activating the Ca\textsuperscript{2+} channels, pumps and exchangers to promote differentiation and growth of metastatic lung cancer cells (MLCCs) in bone.
In regardless of RANKL, TGFβ, a bone resorption-derived factor, enhances the PTHrP expression in tumor cells and OBs, thereby promoting osteolysis [124]. Specifically, TGFβ-mediated signaling pathway activating a couple of important intracellular cascades, consisting of MAPK, PI3K/Akt, and Rho-like GTPase signaling cascades [125], critically acts as a driver of tumor progression and metastasis [126]. Importantly, the tumor cells could significantly produce not only the ILs such as IL-6, IL-8, and IL-11, required for osteoclastogenesis [127], but also strengthen the expression of CXCR4 and CXCR7, establishing a “fertile soil” that accelerates tumor cells to adhere to bone matrix and thrive in bone [128, 129] (Figure 1).

**Conclusion**

In this article, I have reviewed the role of Ca\(^{2+}\) as a key regulator of lung cancer progression and bone metastasis with the metastatic lung cancer. Principally, Ca\(^{2+}\) signals are intrinsic to all aspects of cancer biology, especially in the metastatic lung cancer with bone metastasis. Therefore, identification of the key Ca\(^{2+}\) channels, pumps and/or exchangers would be beneficial for development of treatment strategies for lung cancer. Unfortunately, the exact mechanisms underlying Ca\(^{2+}\) signaling-mediated regulation of lung cancer progression has been incomprehensively understood. Basically, three major steps of bone metastasis required include (1) migration, (2) adhesion and invasion to bone, and (3) proliferation, growth and metastasis in bone. However, it is unclear whether aberrant changes in Ca\(^{2+}\) signals are one of the primary causes of initiation of lung cancer progression.

To what extent can therapeutic strategies exploit these Ca\(^{2+}\)-regulated processes? Accumulating preclinical and clinical evidence has elucidated the relationship between aberrant Ca\(^{2+}\) signaling and tumor progression. Using the specific blockers of Ca\(^{2+}\) channels, pumps and/or exchangers has demonstrated the significant antitumor effects on lung cancer progression (Table 2), indicating that Ca\(^{2+}\) signaling would be a promising target for novel lung cancer treatments. However, before contemplating such efficacious therapeutic interventions based on pharmacological modulation of Ca\(^{2+}\)-regulators, it is crucial to design more potent and specific, but less off-target drugs targeting Ca\(^{2+}\)-regulators, including Ca\(^{2+}\) channels, pumps and/or exchangers. Therefore, further studies are required to verify the toxicity and pharmacokinetic of such modulators prior to the clinical tests.
Table 2. Summary of the major compounds targeting Ca\(^{2+}\) channels/pumps/exchangers in lung cancer progression

| Ca\(^{2+}\) channel/pump/exchanger | Drug Candidates | Pharmacological effects |
|-----------------------------------|-----------------|-------------------------|
| TRPCs                             | SKF-96365       | Cell cycle arrest at S/G2M phase, and invasive ability in A549 cell line [130] |
|                                   | ATRA            | Proliferative inhibition in A549 cells line [131] |
|                                   | 2-ABP           |                                        |
|                                   | Carvacrol       | Degeneration of cell morphology, and apoptosis in A549 cell line [132, 133] |
|                                   | Capsaicin       | Apoptosis in SCLC cell lines, NCI-H82, NCI-H69 [134] |
|                                   | Tetrahydrocannabinol and cannabidiol | Inhibition of proliferation, epithelial-mesenchymal transition (EMT) and migration in A549, H460 and H1792 lung cancer cell lines [135] |
|                                   | Dexamethasone   | Growth suppression in NSCLC cell lines, A549 and H1299 [136] |
| RyR                               | Compound K      | ER-mediated apoptosis in A549 and SK-MES-1 cell lines [137] |
|                                   | Paclitaxel      | Cell cycle arrest at G2/M phase in A549 cell line [138] |
| IP\(_3\)R3                        | A-Lipoic acid (LA) | Apoptosis in A549 cell line [139] |
|                                   | Curcumin        | Apoptosis in NSCLC cell lines, A549 and H1299 [140] |
| VGCCs                             | Verapamil, Diltiazem, and Nifepine | Cell death in chemoresistant lung cancer cells derived from A549 cell line [141] |
| SERCA                             | 2-deoxy D-glucose and metformin | Apoptosis in A549 cell line [142] |
| Voltage-dependent anion channel (VDAC) | R-Tf-D-LP4 | Apoptosis and inhibition of tumor growth HepG2 and Huh-7 cell lines [143] |

Abbreviations

(Ca\(^{2+}\))\(_{e}\): extracellular Ca\(^{2+}\)
(Ca\(^{2+}\))\(_{i}\): intracellular Ca\(^{2+}\)
Bcl-2: B-cell lymphoma-2
CaM: calmodulin
CaMKII: calmodulin-dependent protein kinase II
CaN: calcineurin
CRAC: Ca\(^{2+}\) release-activated Ca\(^{2+}\) channels
CXCL12: C-X-C motif chemokine ligand 12
CXCR4: C-X-C motif chemokine receptor 4
ECM: extracellular matrix
EGFR: epidermal growth factor receptor
ER/SR: endoplasmic/sarcoplasmic reticulum
ER: endoplasmic reticulum
HSCs: hematopoietic stem cells
IL: interleukin
IP\(_3\): inositol-1,4,5-trisphosphate
IP\(_3\)R: IP\(_3\) receptor
MAMs: mitochondria-associated membranes
MAPK: mitogen-activated protein kinase
MLCC: metastatic lung cancer cell
MPT: mitochondrial permeability transition
NFAT: nuclear factor of activated T cell
NSCLC: non-small cell lung cancer
OBs: osteoblasts
OCs: osteoclasts
Orai1: Ca$^{2+}$ release-activated Ca$^{2+}$ channel protein 1
PI3K: phosphoinositol 3 kinase
PKC: protein kinase C
PLC: phosho lipase C
PM: plasma membrane
PTHrP: parathyroid hormone-related peptide
RANKL: receptor activator of nuclear factor kappa-B ligand
RyR: ryanodine receptor
SCLC: small lung cell lung cancer
SERCA: SR/ER Ca$^{2+}$-ATPase
SOC: store-operated Ca$^{2+}$ channel
SOCE: store-operated Ca$^{2+}$ entry
STIM1: stromal interaction molecule 1
TGFβ: transforming growth factor beta
TRP: transient receptor potential
VGCC: voltage-gated Ca$^{2+}$ channel

**Declarations**

**Author contributions**
The author contributed solely to the work.

**Conflicts of interest**
The author declares that there are no conflicts of interest.

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**Consent to participate**
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