Inositol 1, 4, 5-trisphosphate Receptor in Cancer: Good Cop or Bad Cop?

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Abstract:

Calcium signaling is very complex and intimately linked with cell survival and physiological form of cell death. Endoplasmic reticulum is the main intracellular storage of Ca\(^{2+}\) and release of Ca\(^{2+}\) is controlled by ER membrane localized IP\(_3\)-Rs and RYRs. These receptors regulate the transfer of Ca\(^{2+}\) from ER to mitochondria via transport protein on mitochondrial membrane which regulates cellular bioenergetics. Altered IP\(_3\)-R activity and/or the remodeling of IP\(_3\)-R-expression profiles may be exploited by cancer cells to promote growth and drug resistance. It can also affect mitochondrial bio-energetics and susceptibility to apoptotic stimuli, thereby enabling the survival of cells with oncogenic features. Three subtypes of IP\(_3\)-R (IP\(_3\)-R1, IP\(_3\)-R2, and IP\(_3\)-R3) share basic properties but differ in terms of regulation. To what extent they contribute to complex Ca\(^{2+}\) signaling in cancer cells remains largely unknown. Understanding the detailed molecular regulation of IP\(_3\)-Rs will be important to develop novel therapeutic strategies to target cancer cells through their deregulated Ca\(^{2+}\)-signaling machinery. Here we will review the role of IP\(_3\)-R in different cancer as a biomarker or druggable target.

Introduction

Cancer development results from excessive proliferation and uncontrolled cell growth often combined with inhibition of cell apoptosis [1-3]. Calcium ion is a ubiquitous signaling agent controlling numerous cellular processes involved in the regulation of cell growth and cell death [4-6]. In numerous cancer cell lines, interfering with either inositol 1,4,5-trisphosphate (IP\(_3\))-dependent Ca\(^{2+}\) signalling or with the Ca\(^{2+}\) entry leads to the arrest of proliferation [2,7]. Ca\(^{2+}\) homeostasis involves both regulation of Ca\(^{2+}\) entry through different Ca\(^{2+}\)-channels in the plasma membrane and Ca\(^{2+}\) release from internal stores [3]. Endoplasmic reticulum (ER) is the main Ca\(^{2+}\) storage organelle (IP\(_3\)) receptors (IP\(_3\)-Rs) and ryanodine receptors (RYRs) [8,9].

IP\(_3\)-Rs are critical Ca\(^{2+}\)-signaling hubs that critically control cell survival, adaptation and death processes and themselves are tightly regulated by proto-oncogenes and tumor-suppressor proteins [10,11]. When Ca\(^{2+}\) is released from ER by IP\(_3\)-R calcium release channel, it is transported across the mitochondrial membrane by the uniporter calcium channel [12, 13]. The ability of calcium to be taken up by mitochondria is facilitated by the close proximity of the two organelles [13,14]. Upon entry into the mitochondria, calcium functions as a cofactor by activating enzymes that are required for the generation of ATP and regulates mitochondrial bioenergetics [14]. Thus, the filling state of the endoplasmic reticulum Ca\(^{2+}\) stores as well as the Ca\(^{2+}\)-flux properties through the IP\(_3\)-R will affect mitochondrial functions. In contrast, blocking these IP\(_3\)-R-mediated Ca\(^{2+}\) signals from the endoplasmic reticulum activate autophagy, a cellular pro-survival stress response or cell death. The mechanism involves the activation of the AMP-activated kinase (AMPK), a mitochondrial sensor that is activated by an increase in the AMP/ATP ratio [15].

Autophagy plays critical functions in maintaining cellular homeostasis and as an adaptive response to cellular stress, has both antitumor and protumor functions [16]. The role of IP\(_3\)-R in apoptosis is well recognized, but its role in autophagy only recently emerged and is therefore much less well understood. IP\(_3\)-Rs can either suppress or promote autophagy depending upon cellular condition [17-19]. Autophagy facilitates glycolysis and mitochondrial oxidative metabolism during Ras mediated oncogenic transformation [20-22]. Differences in energy metabolism between normal and cancer cells are reported and alterations in cellular bioenergetics are one of the hallmarks of cancer [23-25]. The general principles of metabolic control analysis can be effective for cancer management as abnormal energy metabolism and biological disorder are characteristics of tumors [20]. In line with this, increased aerobic glycolysis and elevated oxidative stress are two prominent biochemical features frequently observed in cancer cells, as shown by the Warburg hypothesis [27]. The deregulation of IP\(_3\)-R plays important role in tumor growth, aggressiveness and drug resistance via modulation of different signaling pathway such as autophagy and energy metabolism.
In normal physiological condition IP3Rs display proper activity in response to IP3, enabled by a good balance between mitochondrial bioenergetics and autophagy. In contrast, in cancer cells the overloading of Ca²⁺ leads to aggressive phenotype, resistant to apoptosis and cell survival during therapy. Ca²⁺ flux from the ER to mitochondria may be dampened either by a decrease in the ER Ca²⁺-store content or by a direct inhibition of the IP3R channel which will display increased AMPK to induce cell death of cancer cells by inhibition or activation of autophagy.

**IP₃ₗ and cancer:** A growing number of studies reveal the altered expression of Ca²⁺ channels and pumps in a plethora of human cancers, including breast, ovarian, glioma, liver, pancreatic, prostate, melanoma, colon, lung, bladder, thyroid, and oral cancer [29-41]. Changes in transient receptor potential channels, voltage-gated Ca²⁺ channels, store-operated Ca²⁺ channels, plasma membrane Ca²⁺ ATPases, intracellular Ca²⁺ release channels, sarco- and endoplasmic reticulum ATPases and secretory pathway ATPases, have been also reported [29, 42]. However, in many cases, the mapping and understanding of the integrated remodeling of the Ca²⁺ signaling toolbox is lacking and the physiological role and importance of these concerted alterations in intracellular Ca²⁺ signaling processes for the biology of the tumor cells and its microenvironment is often poorly understood [29]. It will be critical to understand how the different hallmarks of cancer are influenced or controlled by deregulated Ca²⁺-signaling events, including altered IP₃ₗ function. IP₃ₗ protein subtypes (IP₃ₗ1, IP₃ₗ2 and IP₃ₗ3) are encoded by three different genes in mammals, however the resulting proteins share high similarity in their primary sequences and are expressed to varying degrees in different cell types [19]. Altered IP3R activity and/or the remodeling of IP3R-expression profiles may be exploited by cancer cells to promote their survival, growth, proliferation and migration [28, 43]. Their altered expression profile and function will be important to map and understand how these processes help to survive cancer cells their on-going proapoptotic stress. Fig. 2 describes the various roles of IP₃ₗs in different types of cancer.

**IP₃ₗ and Breast cancer:**

Breast cancer is one of the most common human malignancies and the second leading cause of cancer-related deaths in women, and its incidence in the developing world is on the rise [44-46]. Breast cancer represents approximately 30% of newly diagnosed cancers each year [47, 48]. Three subtypes of IP₃ₗs are expressed in breast cancer cells and intracellular Ca²⁺ release through these channels plays a role in the control of the growth of these cells [30, 43]. Multiple ligands are known to be able to elicit the production of IP₃ and the activation of IP₃ₗs in human breast cells and, among them, adenosine triphosphate (ATP). Numerous works have well described the ATP transduction in breast cancer cell lines [49, 50]. The released ATP binds two types of membrane-bound P2 receptors (P2Rs): ligand-gated P2X receptors, and G protein-coupled P2Y receptors [51-53]. Moreover, IP₃ₗ3 was able to modulate the spatiotemporal pattern of intracellular Ca²⁺ signals induced by ATP [54]. Furthermore, the decrease of its expression level changes the Ca²⁺ signal profile from a plateau-type to a sinusoidal oscillatory-shaped signal which is in favour of a diminution of MCF-7 cell proliferation. IP₃ₗ3 is the unique isoform whose expression is up-regulated in response to 17-beta estradiol (E₂) treatment in MCF-7 cells. Interestingly, this specific effect is able to positively regulate the E₂-induced proliferation of oestrogen-dependent MCF-7 cell line that does not require estrogen receptor (non-genomic signaling) [43, 55, 56]. IP₃ₗ3 interacts with both CyA and CyB and phosphorylation of IP₃ₗs by Cy/cdk complexes provide a novel mechanism of regulating intracellular Ca²⁺ release signaling in breast cancer [57].
K+ channels BKCa channels are also involved in human breast cancer cell proliferation. This effect was impaired when the expression of BKCa and/or IP3R3 has been reduced by specific small interfering RNAs (siRNAs) [49].

Blocking of IP3R (chemical inhibitors such as xestospongin or 2-Aminoethoxydiphenyl borate or siRNA) could also regulate autophagy and energy metabolism in breast cancer therapeutic approach. Further preclinical and clinical studies are warranted to investigate the blockade of IP3R for induction of autophagy in breast cancer. As IP3R antagonist/agonist may have multiple beneficial influences on breast cancer cells, making analogues of the most potent molecule for developing synthetic series with rational drug design approach could pay rich dividends in breast cancer therapy. Actein alters the activity of the IP3R receptor and may be worthwhile to explore to prevent and treat breast cancer [58].

In breast cancer cells, epithelial-mesenchymal transition (EMT) facilitates invasion and metastasis formation, and has also been linked to the acquisition of a stem cell-like phenotype, anchorage-independent growth and chemoresistance in cancer cell lines/clinical samples [59-62]. EMT in breast cancer cells is associated with altered store-operated calcium influx and changes in calcium signalling as analyzed by changes in gene expression of IP3R1 and IP3R3 [49, 59]. Although IP3R expression as a biomarker of breast cancer may have prognostic limitations, it may serve as a drug target for advanced breast cancer, since there are currently few specific therapies that address the primary mediators of metastases in breast cancer.

**IP3R and Gastric cancer**

Higher expression of IP3R3 has been detected in gastric cancer cell line established from the metastasis to the peritoneal cavity (SNU-5, SNU-16, SNU-620, KATO-III and GT3TKB) and lesser IP3R expression in primary main tumors (SNU-1) by differential gene expression method, suggesting that IP3R3 could serve important functions in pathophysiological conditions of gastric cancer [63]. IP3R1 and 2 are only weakly or not expressed in these cells. The antagonist of IP3R, 2APB, inhibited cell proliferation and induced apoptosis in gastric cancer cells from malignant ascites at concentrations of 100nM to 100µM in a dose-dependent manner [63]. On the other hand, 2APB showed a weak effect on other gastric cancer cells established from primary tumors (SNU1), lymph node metastases or liver metastases (MKN1 or 74), melanoma cell lines Met5A and myeloid leukemia HL60 cells. IP3R3 specifically involved in gastric cancer peritoneal dissemination and could be a molecular target of the peritoneal dissemination of gastric cancer. However, the role of the IP3 signaling pathway in the peritoneal dissemination of gastric cancer is still unclear.

**IP3R and Colorectal cancer**

Colorectal cancer is the third leading cause of cancer death among both men and women in the US [64]. Moreover, nearly one million new cases are reported annually worldwide, and this malignancy accounts for almost half a million deaths every year. The efforts to identify new etiologic and prognostic factors have enabled us to predict the clinical outcome of colorectal cancer patients [65]. A major focus has been on genetic and epigenetic alterations that result in colon cancer, including microsatellite and chromosome instability [66]. The epithelium of colon and small intestine is constantly undergoing renewal. Calcium is considered a chemoprotective agent against colon cancer [67, 68]. In vitro studies have demonstrated that maintenance of human colon carcinoma cells in Ca2+-free medium results in increased proliferation of cells that are loosely attached to the substratum [69]. When [Ca2+] (a final concentration of 1.4 mM) is included in the culture medium, cell growth is inhibited, and the cells take on a flattened appearance and behave as a cohesive epithelial unit [69]. In line with these observations, epidemiological studies reveal that high dietary calcium ingestion reduces risks for development of colorectal cancer by inhibiting cell growth and promoting epithelial cell differentiation [70]. IP3R is the principal intracellular Ca2+ release channel in epithelia. Loss of expression of IP3R3 occurs in a range of cholestatic disorders, and evidence suggests that this loss of expression may be directly responsible for impaired ductular secretion [71, 72]. Increased IP3R expression is associated with aggressiveness of colorectal carcinoma [73]. Strong IP3R1 and IP3R2 immunostaining were observed in both normal mucosa and colorectal cancer cells. In contrast, IP3R3 was found only in invading colon cancer cells as prognostic predictor in patients. IP3R3 conferred a survival advantage by inhibiting apoptosis in colon cancer-derived cells, suggesting a pathophysiological basis for this novel biomarker. In colorectal cancer oncogenic K-Ras inhibits Ca2+ release from the ER via modification of IP3R subtypes, reduces ER Ca2+ levels and suppresses Cr2+ flux to the mitochondria [74]. The suppression of Ca2+ signaling is a common response to naturally occurring levels of K-RasG13D and may contribute to a survival advantage during oncogenic transformation and loss of K-RasG13D could induce apoptosis in colon cancer [74].

**IP3R and insulinomas:**

IP3R has been shown to be expressed in purified rat and mouse pancreatic B-cells as well as in human insulinoma cells. Among the subtypes of IP3R, mRNA I, II, and III, IP3R-I mRNA was expressed in mouse islets and the β-cell line BTC3, analyzed by reverse transcriptase–polymerase chain reaction. IP3R-II and -III mRNAs were expressed at similar levels in mouse islets, but neither isoform was detected in BTC3 cells. Insulin release is known to be dependent on Ca2+ as removal of Ca2+ abrogates sustained secondary phase of glucose-induced insulin release.
Elevated levels of [Ca\(^{2+}\)]\(_{o}\) itself could also stimulate insulin release without the presence of any secretagogue [76]. Melatonin inhibits insulin secretion in the pancreatic islets of the rat and in rat insulinoma INS1 cells via Gi-protein-coupled MT1 receptors and the cyclic adenosine 3',5'-monophosphate pathway. However, IP\(_3\)R pathway is involved in the insulin secretory response as well, and the melatonin signal may play a part in its regulation.

**IP\(_3\)R and Bcl-2 dependent cancer:**

Altered Bcl-2 biology has been implicated in a large number of cancer cells, including B-cell lymphomas like diffuse large B-cell lymphoma (DL-BCL) and chronic lymphocytic leukemia (CLL) [77-80]. In many cases, Bcl-2 is upregulated, increasing the resistance of the cancer cell toward pro-apoptotic signals like oncogenic stress or genomic instability and thus promoting their survival [81, 82]. The relative IP\(_3\)R expression level was an important determinant for the apoptotic response of these cells, and correlated with the ability of protease-resistant form of the peptide (TAT-IDPS) to trigger pro-apoptotic IP\(_3\)R-mediated Ca\(^{2+}\) release [83]. The disruption of Bcl-2 binding to IP\(_3\)Rs was particularly effective in cancer cells with high levels of IP\(_3\)R2 [84]. The presence of IP\(_3\)R2 rendered these cells vulnerable toward ongoing IP\(_3\) signaling, for example, like during chronic activation, whereas cells expressing relatively low levels of IP\(_3\)R2 were much less sensitive. Such a correlation was not observed for the two other IP\(_3\)R isoforms (IP\(_3\)R1 and IP\(_3\)R3) and IP\(_3\)R isoform as a determinant for the sensitivity to cell death in Bcl-2-dependent cancer cell lines [83].

**IP\(_3\)R and Lung cancer:**

Lung cancer is the leading cause of cancer death in the industrial nations [85]. Despite recent advances, therapeutic regimens support quality of life but frequently fail to increase long term survival. One of the main reasons for the failure of therapeutic regimens is the fact that cancer cells originate from normal cells and therefore possess similar characteristics. Thus, knowledge of the differences in the cellular physiology between malignant and non-malignant cells is crucial for the development of more successful treatments. Calcium is particularly important for the regulation of proliferation and apoptosis and the imbalance of cell growth and cell death finally leads to lung cancer. The increased IP\(_3\)R expression was analyzed in H1339 and HCC cells which is in agreement with in vivo data obtained from patients with resectable NSCLC [86, 87].

**Conclusion:** Cancer cells likely undergo a complete remodeling of their Ca\(^{2+}\) transport mechanisms both at the plasma membrane and at the intracellular organelles, including the endoplasmic reticulum and the Golgi apparatus. The link between Ca\(^{2+}\), ER stress, and autophagy relies on the modulation of IP3R. This receptor releases Ca\(^{2+}\) from ER stores in response to different cellular signals, although it could also play additional functions derived from its ability to interact with different proteins, including members of the Bcl-2 family [88]. Bcl-2 directly interacts with IP3Rs to inhibit IP3-dependent calcium flux [89]. XesB or lithium-induced decrease of myo-IP3 levels, and disrupts the interaction of IP3R with Beclin-1 and bcl-2 [90-93]. Altered expression of IP3R in different types of cancer could be responsible for metastasis process and it can be used as biomarker. Targeting of IP3R with chemical inhibitors could be a successful treatment for advanced metastatic cancer. Further investigation is nevertheless necessary to clarify whether this mechanism participates in the activation of autophagy and energy metabolism in response to ER stress.

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