Biochimica et Biophysica Acta xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Calcium remodeling in colorectal cancer☆

Carlos Villalobos *, Diego Sobradillo, Miriam Hernández-Morales, Lucía Núñez

Instituto de Biología y Genética Molecular (IBGM), Universidad de Valladolid y Consejo Superior de Investigaciones Científicas (CSIC), 47003 Valladolid, Spain

ARTICLE INFO

Article history:
Received 31 October 2016
Received in revised form 5 January 2017
Accepted 7 January 2017
Available online xxxx

Keywords:
Colorectal cancer
Store-operated Ca^{2+} entry
Orai1
TRPC1
STIM1
STIM2
Aspirin
Non-steroidal anti-inflammatory drugs

ABSTRACT

Colorectal cancer (CRC) is the third most frequent form of cancer and the fourth leading cause of cancer-related death in the world. Basic and clinical data indicate that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) may prevent colon cancer but mechanisms remain unknown. Aspirin metabolite salicylate and other NSAIDs may inhibit tumor cell growth acting on store-operated Ca^{2+} entry (SOCE), suggesting an important role for this pathway in CRC. Consistently, SOCE is emerging as a novel player in different forms of cancer, including CRC. SOCE and store-operated currents (SOCs) are dramatically enhanced in CRC while Ca^{2+} stores are partially empty in CRC cells. These features may contribute to CRC hallmarks including enhanced cell proliferation, migration, invasion and survival. At the molecular level, enhanced SOCE and depleted stores are mediated by overexpression of Orai1, Stromal interaction protein 1 (STIM1) and Transient receptor protein channel 1 (TRPC1) and downregulation of STIM2. In normal colonic cells, SOCE is mediated by Ca^{2+}-release activated Ca^{2+} channels made of STIM1, STIM2 and Orai1. In CRC cells, SOCE is mediated by different store-operated currents (SOCs) driven by STIM1, Orai1 and TRPC1. Loss of STIM2 contributes to depletion of Ca^{2+} stores and enhanced resistance to cell death in CRC cells. Thus, SOCE is a novel key player in CRC and inhibition by salicylate and other NSAIDs may contribute to explain chemoprevention activity.

Summary: Colorectal cancer (CRC) is the third most frequent form of cancer worldwide. Recent evidence suggests that intracellular Ca^{2+} remodeling may contribute to cancer hallmarks. In addition, aspirin and other NSAIDs might prevent CRC acting on remodeled Ca^{2+} entry pathways. In this review, we will briefly describe 1) the players involved in intracellular Ca^{2+} homeostasis with a particular emphasis on the mechanisms involved in SOCE activation and inactivation, 2) the evidence that aspirin metabolite salicylate and other NSAIDs inhibits tumor cell growth acting on SOCE, 3) evidences on the remodeling of intracellular Ca^{2+} in cancer with a particular emphasis in SOCE, 4) the remodeling of SOCE and Ca^{2+} store content in CRC and, finally, 5) the molecular basis of Ca^{2+} remodeling in CRC. This article is part of a Special Issue entitled: ECS Meeting edited by Claus Heizmann, Joachim Krebs and Jacques Haiech.

1. Introduction

Colorectal cancer (CRC) is one of the most frequent forms of cancer and the fourth leading cause of cancer-related death in the world, with an estimated incidence of 1.250.000 new cases every year and more than 600.000 deaths annually. Unfortunately, despite new developments of targeted therapy and other improvements, the prognosis for patients with metastatic CRC remains very limited [1]. This reality highlights the need for efficient chemoprevention and new therapies. Interestingly, there is a considerable body of pre-clinical, epidemiological and randomized data supporting the hypothesis that aspirin prevents CRC and has the potential to be an effective adjuvant cancer therapy [2]. A series of clinical trials show that aspirin reduces the risk of colorectal adenomas [3,4] but the action mechanism remains elusive. Although anti-inflammatory activity may contribute to chemoprevention, evidence suggests that action mechanism is largely independent of anti-inflammatory activity. Aspirin irreversibly inhibits cyclooxygenase 1 (COX-1) activity by acetylating the enzyme at Ser530 and inducible COX-2 at Ser516. In vivo, aspirin is quickly deacetylated to salicylic acid, which remains in plasma for much longer. Salicylic acid does not directly inhibit COX activity because it lacks the acetyl group, although it may inhibit COX-2 gene expression at the transcription level [5]. Thus, inhibition of both COX-2 activity and COX-2 gene expression has been proposed to contribute to the anti-tumoral effects of aspirin. However, aspirin and COX-2 inhibitors block proliferation through a prostaglandin-independent pathway as they inhibit cell proliferation in both COX-2 expressing cells (HT29 and HCA-7) as well as in cells not expressing COX-2 (SW480 and HTC-116) [6,7]. In the same line, the anti-proliferative effects of NSAIDs are independent of the level of COX-2 expression [8] or prostaglandin E2 production [9] but related to cell

☆ This article is part of a Special Issue entitled: ECS Meeting edited by Claus Heizmann, Joachim Krebs and Jacques Haiech.

* Corresponding author at: Instituto de Biología y Genética Molecular (CSIC), c/Sanz y Forés 3, 47003 Valladolid, Spain.
E-mail address: carlosv@ibgm.uva.es (C. Villalobos).

http://dx.doi.org/10.1016/j.bbamcr.2017.01.005
0167-4889/

cycle quiescence. Microarray analysis has shown that aspirin represses many cell-cycle-related genes and modulates multiple signaling pathways suggesting that an early mitotic signal may be the key target for the anti-proliferative effect [10].

A few years ago, we reported that salicylate, the major aspirin metabolite, inhibits CRC cell proliferation acting on store-operated Ca^{2+} entry (SOCE), a Ca^{2+} entry pathway involved in cell proliferation in different cell types [11,12]. This pathway is activated by physiological agonists that induce phospholipase C activity and synthesis of IP_3. In turn, IP_3 activates ligand-gated, Ca^{2+} release channels at the endoplasmic reticulum (ER) inducing the emptying of the intracellular Ca^{2+} stores and SOCE activation. This pathway, first envisioned by James W. Putney [13,14], is responsible for agonist-induced Ca^{2+} entry in most types of cells, being particularly abundant and relevant in non-excitable cells that lack voltage-gated Ca^{2+} influx [15]. The functional role of this pathway was originally believed to be intended solely to support the refilling of intracellular Ca^{2+} stores after cell stimulation. However, it is becoming increasingly clear that SOCE subserves multiple physiological roles in different cells types including control of cell proliferation [15]. This view is supported by a large series of papers showing that pharmacological inhibition of this pathway or knockdown of molecular players involved results almost invariably in inhibition of cell proliferation [15]. Therefore, as this pathway is prevented by the aspirin metabolite salicylate, it follows that many pharmacological actions attributed to aspirin and dietary salicylates, including the prevention of CRC, could be mediated in fact by inhibition of this important Ca^{2+} entry pathway.

Recent data suggest an unexpected role for SOCE in cancer including CRC [16–19]. Fortunately, the molecular basis of SOCE emerged recently [20,21], thus providing the tools to investigate the role of SOCE in cancer on solid grounds. Soon, it become clear that many of the molecular players involved in SOCE are expressed differentially in multiple tumor cells and may contribute significantly to some cancer hallmarks in a series of forms of cancer including hepatoma, glioblastoma, breast cancer and CRC [22–24]. Some of these molecular players are presently under scrutiny to ascertain whether they may actually be considered as markers of cancer progression. Moreover, even more recent evidence indicate that mutations in oncogenes and tumor suppressors commonly found in different forms of cancer are able to promote changes in intracellular Ca^{2+} homeostasis and contribute to Ca^{2+} remodeling in cancer [25].

In this review, we will briefly describe 1) the players involved in intracellular Ca^{2+} homeostasis with a particular emphasis on the mechanisms involved in SOCE activation and inactivation, 2) the evidence that aspirin metabolite salicylate and other NSAIDs inhibits tumor cell growth acting on SOCE, 3) evidences on the remodeling of intracellular Ca^{2+} in cancer with a particular emphasis in SOCE, 4) the remodeling of SOCE and Ca^{2+} store content in CRC and, finally, 5) the molecular basis of Ca^{2+} remodeling in CRC.

2. Intracellular Ca^{2+} homeostasis: the players

Intracellular Ca^{2+} is a versatile second messenger involved in the control of many different cell and physiological functions. Ca^{2+} is not synthesized or metabolized inside cells like the rest of second messengers. Instead, it is transported down and up electrochemical gradients through specific channels and pumps, respectively, located at plasma membranes or endomembranes of the ER, mitochondria and other organelles. The cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) is very low, in the nM range, so that small changes in Ca^{2+} channel conductance promote quick changes in $[Ca^{2+}]_{cyt}$. These changes are sensed by calmodulin and by many other Ca^{2+}-operated enzymes that start a signaling cascade or promote cell responses in virtually all types of cells. Changes in $[Ca^{2+}]_{cyt}$ may be global in the whole cell or restricted in time and space resulting in elementary events named Ca^{2+} microdomains that regulate cellular functions restricted in special subcellular regions such as exocytosis in plasma membrane, cell respiration and ATP synthesis in mitochondria or gene transcription in the nucleus. Whereas Ca^{2+} pumps and transporters contribute largely to the maintenance of basal or resting $[Ca^{2+}]_{cyt}$ and to the recovery of basal $[Ca^{2+}]_{cyt}$ after stimulation, most increases in $[Ca^{2+}]_{cyt}$, are induced by activation of Ca^{2+} channels at the plasma membrane and Ca^{2+} release channels at the ER. IP_3 and ryanodin receptors are ligand-gated Ca^{2+} channels involved in Ca^{2+} release from stores after agonist stimulation. These Ca^{2+} release channels induce transient rises in $[Ca^{2+}]_{cyt}$ but their activity may secondary activate Ca^{2+} channels in plasma membrane that are gated by emptying of intracellular Ca^{2+} stores, the so-called SOCE or capacitative Ca^{2+} entry [15].

In mitochondria, the main Ca^{2+} channel is the mitochondrial Ca^{2+} uniporter (MCU), a Ca^{2+}-activated, Ca^{2+} channel recently characterized at the molecular level [26,27]. Activation of this channel requires large $[Ca^{2+}]_{cyt}$. At variance with the rest of channels, activation of the MCU removes Ca^{2+} from cytosol into mitochondria and helps clearing large cytosolic Ca^{2+} loads [28]. Resting mitochondrial $[Ca^{2+}]$ is similar to resting cytosolic $[Ca^{2+}]$. However, Ca^{2+} influx into mitochondria is empowered by the huge mitochondrial potential ($\Delta\Psi$), close to −180 mV and negative inside the inner mitochondrial membrane [12]. However, the mitochondrial Ca^{2+} uniporter (MCU), the channel responsible for mitochondrial Ca^{2+} uptake is normally closed, thus not allowing the influx of Ca^{2+} into mitochondria unless surrounding Ca^{2+} is large enough to activate this Ca^{2+} operated, Ca^{2+} channel. Ca^{2+} microdomains at the mouth of the MCU can be formed during activation of voltage-gated Ca^{2+} channels in excitable cells like chromaffin [29] and anterior pituitary cells [30]. In addition, large Ca^{2+} microdomains may be formed during release of Ca^{2+} from the ER at close contact sites between the ER and mitochondria enabling efficient ER-mitochondria cross talk [31].

At the plasma membrane there are many different types of Ca^{2+} channels, including receptor-operated Ca^{2+} channels (ROCCs) and voltage-operated Ca^{2+} channels (VOCCs) widely expressed in excitable cells together with voltage-independent channels particularly relevant in the non-excitable cells. In these latter cells, the most important Ca^{2+} entry pathway is the above mentioned SOCE. As many other Ca^{2+} channels, the Ca^{2+} channel responsible for SOCE is usually inactivated by Ca^{2+} leading to a transient Ca^{2+} entry. This mechanism is prevented by mitochondria located nearby these channels that are able to sense high Ca^{2+} microdomains and remove Ca^{2+} to prevent the Ca^{2+}-dependent inactivation of these channels [32,33] (Fig. 1). Thus, mitochondria play a pivotal role in sustaining Ca^{2+} signals initiated by SOCE. This is the case, for instance, of the activation of the nuclear factor of activated T cells (NFAT) during the immunological synapse. T cell receptor activation induced by antigen presentation, promotes Ca^{2+} release and Icrac activation in T cells. Nearby located mitochondria take up entering Ca^{2+} and prevent the slow, Ca^{2+}-dependent Icrac inactivation, thus leading to a sustained entry of Ca^{2+}. The sustained and moderate increase in $[Ca^{2+}]_{cyt}$ activates calcineurin, a Ca^{2+}-dependent phosphatase that removes NFAT phosphorylation revealing an NFAT nuclear import signal. The sustained presence of NFAT in the nucleus allows the expression of interleukin 2 that promotes finally the clonal expansion of the activated T cell [34]. Therefore, the role of mitochondria in removing the Ca^{2+}-dependent inactivation of Icrac channels is responsible for sustaining SOCE and cell proliferation in T cells. We have shown that salicylate, the major aspirin metabolite, depolarizes partially mitochondria and limits largely the ability of mitochondria of Jurkat T cells to take up Ca^{2+}, thus promoting SOCE inactivation and inhibition of cell proliferation in T cells [11].

Since the first description of SOCE by James W. Putney [13,14], the molecular basis of SOCE remained elusive for nearly 20 years. However, the molecular players involved in SOCE began to be crack after the discovery of the TRP superfamily of ion channels first, and the subsequent discovery of STIM and Orai proteins [20,21]. At the molecular level, SOCE starts with the emptying of intracellular Ca^{2+} stores from resting

Please cite this article as: C. Villalobos, et al., Calcium remodeling in colorectal cancer, Biochim. Biophys. Acta (2017), http://dx.doi.org/10.1016/j.bbamcr.2017.01.005