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Review

CRAC channels, calcium, and cancer in light of the driver and passenger concept☆

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ABSTRACT

Advances in next-generation sequencing allow very comprehensive analyses of large numbers of cancer genomes leading to an increasingly better characterization and classification of cancers. Comparing genomic data predicts candidate genes driving development, growth, or metastasis of cancer. Cancer driver genes are defined as genes whose mutations are causally implicated in oncogenesis whereas passenger mutations are defined as not being oncogenic. Currently, a list of several hundred cancer driver mutations is discussed including prominent members like TP53, BRAF, NRAS, or NF1. According to the vast literature on Ca^{2+} and cancer, Ca^{2+} signals and the underlying Ca^{2+} channels and transporters certainly influence the development, growth, and metastasis of many cancers. In this review, I focus on the calcium release-activated calcium (CRAC) channel genes STIM and Orai and their role for cancer development, growth, and metastasis. STIM and Orai genes are being discussed in the context of current cancer concepts with a focus on the driver-passenger hypothesis. One result of this discussion is the hypothesis that a driver analysis of Ca^{2+} homeostasis-related genes should not be carried out by looking at isolated genes. Rather a pool of "Ca^{2+} genes" might be considered to act as one potential cancer driver. This article is part of a Special Issue entitled: Calcium and Cell Fate edited by Jacques Haiech, Claus Heizmann, and Joachim Krebs.

1. Introduction

Uncontrolled cancer growth and metastasis would not be nearly as devastating if our immune system were able to quickly recognize and eliminate cells that have undergone transformation to malignancy in every single case. Eliminating cancer cells is therefore one of the major challenges of immune cells. Cytotoxic T lymphocytes and natural killer cells are in principle able to detect cancer cells although through distinct surface receptor interactions but share rather similar mechanisms to eliminate cancer cells by releasing perforin and/or granzymes or by employing the Fas/FasL system. Two major problems of immune therapy are the recognition of cancer cells and the potentially pro-oncogenic tumor microenvironment which can result in the inhibition of effective immune responses or can result in a misuse of the immune system for cancer growth. However, recent progress in different areas has led to developments in the field of cancer immune interactions which already facilitate and should further facilitate the optimization of the immune interference against cancer:

1) A much better definition of tumor driver and passenger genes has been achieved with the help of array technology and next-generation sequencing in combination with big data bioninformatics analyses. Cancer driver gene analyses have been performed by different groups including Vogelstein et al. [1] and Tramborero et al. [2]. Most of the somatic mutations identified in a single cancer sample are, however, only passenger mutations. The definition of molecular signatures in human cancers has also quickly advanced with better sequencing technologies [3].

2) Cancer therapies interfering with immune checkpoints (mainly CTLA-4 and PD-1) have been developed during the last few years.

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CTLA-4 and PD-1 act as brakes on effector mechanisms of cytotoxic cells. Interfering with their inhibitory function by antibodies thereby releasing immune effector mechanisms has been successfully used in cancer therapies, for instance against melanoma [4,5].

3) Improvements in the adoptive immune cell transfer are currently being used to enhance the effective immune response. One major advance is for instance the engineering of chimeric antigen receptors (CARs) against defined tumor-associated antigens [6,7], which are successfully being used in cancer therapy.

Since Ca^{2+} ions play an exceptionally important role for cell proliferation, apoptosis, and function [8], it is no surprise that many publications address the role of Ca^{2+} for tumor development and progression. CRAC channels are a major Ca^{2+} influx pathway in both cancer and immune cells and the identification of STIM and Orai proteins as the core of this influx pathway has greatly facilitated the analysis of their potential role in cancer development and progression. Since Ca^{2+} is involved in both, cancer growth and its elimination by cytotoxic immune cells, Ca^{2+} signaling should not be analyzed in isolation in cancer or immune cells, since any interference with Ca^{2+} signaling in the tumor may affect both, cancer cells and immune cells. Interestingly, there is also a link between Ca^{2+} signaling and CTLA-4 activation, which is one of the main receptors inhibited during checkpoint therapy [4,5]: CTLA-4 activation inhibits CRAC channel activity potentially involving Orai3 and STIM2 [9].

Here we review the role of CRAC channels in cancer cells including development, growth, and metastasis, thereby complementing our review "on the role of Ca^{2+}-dependent killing of cancer cells by CTL and NK cells" in a previous special issue [10].

2. 25 years CRAC channels and 10 years Orai channels

About 25 years ago, calcium release-activated calcium (CRAC) channels were reported the first time following store depletion in mast cells [11,12] and T cells [13]. These initial papers from the Penner and Lewis laboratories mark the beginning of very broad research by many groups focusing on CRAC channels' fundamental properties, physiological and pathophysiological functions, and its molecular identity. The identification of I_{crac} also further advanced the concept of store-operated Ca^{2+} (SOC) entry or as it was initially called by Putney, capacitative Ca^{2+} entry [14]. Many of the important properties of CRAC channels like their extremely high Ca^{2+} selectivity [15], Ca^{2+}-dependent inactivation [16,17], and single-channel conductance were already quite well defined in the initial three papers [11–13]. The growing know-how on these and other properties and on physiological and later also pathophysiological functions of CRAC channels were reviewed many times in the following 10 years including very detailed reviews by some of leading experts in the field [18–21]. Only about 10 years ago, the molecular backbone of CRAC channels was finally unmasked by first identifying its activators STIM1 and STIM2 [22–24], which report the filling state of the Ca^{2+} stores to the plasma membrane, and second its pore forming units Orai1, Orai2, or Orai3 [25–27]. Unexpectedly, Orai channels most likely form hexameric but not tetrameric channels as might have been expected from voltage-gated Ca^{2+} and K^+ channels [28].

3. STIM and Orai in cancer

The importance of Ca^{2+} as a target for disease treatment in humans was already recognized about 135 years ago [29] when calcium chloride was applied during chronic pulmonary phthisis (old for tuberculosis). The first link between Ca^{2+} and cancer which I am aware of was published almost 70 years ago [30,31]. Among the currently 1800 publications in PubMed having the term calcium (or Ca^{2+}) and cancer (or tumor) in the publication title (180.000 with keywords calcium and cancer), a linear increase of 30 in 2001 to 90 in 2014 is observed.

From the many published data, it is quite clear that Ca^{2+} influences cancer cell growth and proliferation as would be expected since Ca^{2+} is clearly linked to (cancer) cell proliferation. Simple conclusions should however not be made, since both in vitro and in vivo measurements have revealed many different effects of calcium ranging from growth inhibition to increased cell proliferation of cancer cells (for reviews, see [32–38]). According to a meta-analysis of controlled and randomized trials, orally administered Ca^{2+} supplements do not change the total cancer risk over 4 years [39]; however, as the authors state, "the meta-analysis lacked power to detect very small effects, or those with a longer latency." In primary human $CD4^+$ T cells, we have found a very good correlation between intracellular Ca^{2+} amplitudes mediated through CRAC channels and cell proliferation [40]. Unexpectedly, $CD4^+$ cells from Orai1$^{-/-}$ mice showed normal proliferation but reduced activated T cell death [41]. The finding that Orai1 is not required for mouse T cell proliferation does not necessarily mean that CRAC channels are not required because the remaining Orai2 and Orai3 activity might provide sufficient Ca^{2+} entry for T cell proliferation in the absence of Orai1. In my opinion, this is a likely scenario because 100 nM of the CRAC channels blocker BTP2 inhibits T cell proliferation by only 50% but Ca^{2+} signals by more than 90% over extended times [42]. This means that only very little Ca^{2+} entry is required for T cell proliferation. However, a difference between human and mouse T cells' Ca^{2+} dependence of proliferation cannot be ruled out.

The molecular identification of STIM and Orai as the CRAC channel genes opened up different ways to analyze the potential importance of CRAC channels in cancer. From the 250 publications about STIM, Orai, and cancer, most of which were published during the last 3 years, I have deduced the findings presented in Table 1. The list includes relevant data on STIM and Orai and their correlation to apoptosis (A), cell cycle progression (C), expression (E), invasion (I), migration (M), metastasis (Mt), proliferation (also tumor growth or progression, P), or survival (also viability, S) of different cancer cells/tissue divided into data from primary human material, mouse material, or in vivo mouse data, or cell lines. From the table, the following conclusion can be drawn:

Orai1 expression is increased in all but one human cancer tissue analyzed: 1) clear cell renal carcinoma (ccRCC) [43]; 2) breast cancer (expression of a certain Orai1 SNP [44]; 3) esophageal squamous cell carcinoma (ESCC), and increased Orai1 expression was found to correlate with survival [45]; 4) glioma samples, and increased Orai1 expression was found to correlate with WHO staging [46]; 5) non-small cell lung cancer (NSCLC) tissue [47]; 6) human melanoma tissues [48,49].

Particularly high Orai1 expression was also found in dermis invading tumor nests and lymph node metastasis [49]. Recently, one of the novel PKCs was reported to control Orai1 function in invasive melanoma [50], picking up the concept of phosphorylation-dependent regulation of I_{crac} in RBL cells [51].

Compared to other cancer tissue, prostate cancer the only exception, in which Orai1 expression was decreased compared to normal tissue but increased compared to hyperplasia [52]. Interestingly, another highly Ca^{2+}-selective ion channel, TRPV6, is upregulated in advanced prostate cancer compared to hyperplasia and its expression even correlates with tumor grading [53]. Accordingly, a clear correlation between TRPV6 overexpression and the rate of Ca^{2+}-dependent cell proliferation was observed [54]. TRPV6 overexpression could thus fulfill the same function in prostate cancer as Orai channels may do for the other cancers tested.

A role of Orai1 for proliferation and migration of cancer cells was tested in clear cell renal carcinoma (ccRCC) [43] and in esophageal squamous cell carcinoma (ESCC) [45], supporting the evidence that higher Orai1 expression is indeed tumorigenic. At large, this picture was also confirmed in the cell lines (Table 1). It can thus be concluded that Orai1 expression is increased in many cancer types and is very likely involved in tumor growth, metastasis, migration, and invasion. In line with this conclusion, genetic polymorphisms of Orai1 were found to be associated with estrogen receptor-positive breast cancer and lymph nodal involvement in a Taiwanese population [44]. Furthermore, Zhan

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