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Novel perspectives in cancer therapy: Targeting ion channels

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ABSTRACT

By controlling ion fluxes at multiple time scales, ion channels shape rapid cell signals, such as action potential and synaptic transmission, as well as much slower processes, such as mitosis and cell migration. As is currently increasingly recognized, a variety of channel types are involved in cancer hallmarks, and regulate specific stages of neoplastic progression. Long-term *in vitro* work has established that inhibition of these ion channels impairs the growth of cancer cells. Recently, these studies have been followed up *in vivo*, hence revealing that ion channels constitute promising pharmacological targets in oncology.

The channel proteins can be often accessed from the extracellular milieu, which allows use of lower drug doses and decrease untoward toxicity. However, because of the central physiological roles exerted by ion channels in excitable cells, other types of side effects may arise, the gravest of which is cardiac arrhythmia. A paradigmatic case is offered by $K_v11.1$ (hERG1) channels. HERG1 blockers attenuate the progression of both hematologic malignancies and solid tumors, but may also lead to the lengthening of the electrocardiographic QT interval, thus predisposing the patient to ventricular arrhythmias. These side effects can be avoided by specifically inhibiting the channel isoforms which are highly expressed in certain tumors, such as $K_v11.1B$ and the neonatal forms of voltage-gated Na^+ channels. Preclinical studies are also being explored in breast and prostate cancer (targeting voltage-gated Na^+ channels), and gliomas (targeting CLC-3). Overall, the possible approaches to improve the efficacy and safety of ion channel targeting in oncology include: (1) the development of specific inhibitors for the channel subtypes expressed in specific tumors; (2) drug delivery into the tumor by using antibodies or nanotechnology-based approaches; (3) combination regimen therapy and (4) blocking specific conformational states of the ion channel. We believe that expanding this relatively neglected field of oncology research might lead to unforeseen therapeutic benefits for cancer patients.

1. Introduction

Various tumors are characterized by a profound genomic instability (Hanahan and Weinberg, 2011), which accounts for the progressive accumulation of mutations. As a consequence, tumor cells acquire, during the process of tumor progression, new genotypic and phenotypic features which give rise to different malignant clones. Tumor progression thus leads to the acquisition of a tumor molecular profile, which is often peculiar to each individual and whose relation with the pathogenic process is difficult to unravel (Weinberg, 2007). Although understanding this molecular diversity can pave the way to develop more personalized therapies, such a goal currently appears difficult to reach. The alternative strategy, typical of classic chemotherapeutics, is to identify biological factors essential to the physiology of cancer cells. Because most of the identified pharmacological targets participate in the cell cycle machinery, which is active in all replicating cells, their inhibition can induce serious side effects. Thus, currently, a main challenge in oncology is to identify alternative biological targets which are either implicated in some aspects of tumor progression and have less widespread physiological roles, or are specific for some tumor type.

Recently, studies focusing on the functional implications of ion channels in the biology of cancer cells have grown into a mature field of research, and the related literature has grown explosively in the last few years (e.g., Becchetti et al., 2013, and references therein). Widespread alterations in the expression and activity of virtually any kind of ion channels have been observed in neoplastic cells, which at the same time mark specific tumor progression stages and regulate their course (Arcangeli et al., 2009). There are two main reasons why such a wide variety of ion channels has

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been found to be implicated in tumor progression. First, ion fluxes regulate most of the cellular processes involved in the neoplastic progression, such as cell volume oscillations (Pedersen et al., 2013; Turner and Sontheimer, 2014) and progression through the mitotic checkpoints (Becchetti, 2011). Under this respect, ion transporters flank ion channels in regulating these properties. The cognate field of ion transporters in cancer has been previously reviewed (Cardone et al., 2005; Lefranc et al., 2008; Harguindey et al., 2009; Hoffmann et al., 2009; Andersen et al., 2014). Second, ion channels have a complex and flexible functional nature, which renders them particularly apt to simultaneously participate in disparate cellular properties. The fast kinetic properties of many ion channels make them regulate multiple processes on rapid time scales [millisecond (ms) to tenths of ms], such as action potentials or synaptic responses. At the same time, the steady-state properties of these channels can regulate complex physiological processes at much slower time scales, ranging from V_{rest} control, to the response of cells to hormones, to the regulation of circadian rhythm. Importantly, ion channels can also exert non-conductive physiological roles, by forming macromolecular complexes with other membrane proteins (Arcangeli and Becchetti, 2006; Pillozzi et al., 2007) or through intracellular domains capable of exerting enzyme functions (Kaczmarek, 2006).

In fact, different types of ion channels are implicated in the so-called hallmarks of cancer (Prevarskaya et al., 2010). These comprise limitless proliferative potential that does not depend on exogenous growth signals, insensitivity to anti-replication signals, avoidance of apoptosis as well as of immunologic response, stimulation of neoangiogenesis, of invasive ability and of tumor-promoting inflammation, as well as the presence of genome instability and of a profound deregulation of cellular energetics (Hanahan and Weinberg, 2011). The effects of ion channels are exerted by a specific control of the cell cycle phases (Becchetti, 2011; Urrego et al., 2014), of the mechanisms sustaining cell survival and apoptosis (Lehen'kyi et al., 2011), angiogenesis (Fiorio Pla et al., 2012), as well as cell migration and invasiveness (Cuddapah and Sontheimer, 2011; Schwab and Stock, 2014) and hence tumor metastasis (Djamgoz and Onkal, 2013).

2. Ion channel expression and functional role in cancer

A comprehensive review of the roles of ion channel in cancer cells would be inappropriate in the present context. For reader's convenience, we will provide a concise overview with no pretension of exhaustion, referring to specific reviews for comprehensive treatment (Lastraioli et al., 2014). Evidence is particularly extensive regarding the expression and function of K^+ channels in a variety of solid tumors and hematologic malignancies (Arcangeli et al., 2009, 2012; D'Amico et al., 2013; Pardo and Stühmer, 2014). Current pertaining studies are aimed at unraveling the contribution of each channel type to cancer progression, which is complicated by the variety of K^+ channels. In brief, cancer cell proliferation is often modulated by voltage-gated K^+ channels (VGKCs), and especially $K_v1.3$, K_v 1.5 (Comes et al., 2013), K_v 10.1 (Hemmerlein et al., 2006; Downie et al., 2008) and K_v 11.1 (Pillozzi et al., 2002; Smith et al., 2002; Wang et al., 2002; Arcangeli, 2005). The special role of K_v 11.1 (also known as ERG1, from *Ether-à-go-go* Related Gene, hERG in humans; Warmke and Ganetzky, 1994) is discussed later. Moreover, Ca^{2+}-dependent K^+ channels, especially $K_{Ca}1.1$ and $K_{Ca}3.1$ (Turner et al., 2014; Grössinger et al., 2014; D'Alessandro et al., 2013) and two-pore (K_{2p}) channels (Cid et al., 2013; Williams et al., 2013), have been shown to exert specific roles. K^+ channels participate in a variety of cellular mechanisms implicated in cell proliferation and cancer progression. We mention the V_m-dependent modulation of transmembrane Ca^{2+} flow (Ouadid-Ahidouch and Ahidouch, 2013), the contribution to the KCl extrusion typical of regulatory volume decrease (Hoffmann et al., 2009), the direct control of cell-adhesion proteins (Arcangeli and Becchetti, 2006), and growth factor receptors (Pillozzi et al., 2007), through the formation of macromolecular complexes in the plasma membrane.

The contribution of Ca^{2+} is manifold; intracellular Ca^{2+} is thought to modulate cell cycle checkpoints in normal and neoplastic cells (Monteith et al., 2007; Becchetti, 2011). Moreover, Ca^{2+} signals regulate apoptosis (Prevarskaya et al., 2014), autophagy (Kondratskyi et al., 2013), and the release of hormones, growth factors and neurotransmitters. The passive Ca^{2+} flux across the plasma membrane is mediated by channels belonging to the well-known voltage-gated superfamily as well as other types of permeation pathways that are currently the focus of an intense investigation (Azimi et al., 2014). Most of the recent evidence in cancer cells concerns these latter Ca^{2+} pathways. In particular, the altered regulation of Ca^{2+} signaling in tumor progression is often related to the Transient Receptor Potential (TRP) channels (Zhang and Barritt, 2006; Bidaux et al., 2007; Hamdollah Zadeh et al., 2008; Fiorio Pla and Gkika, 2013; Morelli et al., 2013; Chen et al., 2014), as well as to the store-operated channels.

K^+, Cl^- and Ca^{2+} channels closely interplay in regulating cell volume, which plays a role in cell proliferation, apoptosis and cell migration. For example, several swelling-activated channels have been implicated in cancer cell physiology, such as the volume-regulated anion channels (VRAC), the swelling-activated K^+ channels TASK-2 and $K_{Ca}1.1$ (hsloBK), and the volume sensitive Ca^{2+}-permeable TRPV4 and TRPM4 (Hoffmann et al., 2009). Efflux of KCl underlies the early volume decrease during apoptosis, as well as regulates the volume changes during glioma cell invasion of the surrounding narrow interstitial spaces (Mamelak et al., 2006; Cuddapah and Sontheimer, 2010, 2011). $K_{Ca}3.1$ is also thought to be implicated in localized cell volume changes occurring during cell migration (Schwab et al., 2007).

The expression of voltage-gated Na^+ channels (VGSCs) has also been observed to increase in many cancer types, including breast, prostate, lung (both small-cell, SCLC, and non-small-cell, NSCLC), cervical cancer, leukemia (reviewed in Arcangeli et al., 2009; Fraser et al., 2014) and mesothelioma (Fulgenzi et al., 2006). In addition, where studied, alternative splice variants (e.g. neonatal forms) have been shown to be preferentially expressed (Fraser et al., 2014). In breast, prostate and NSCLC tumor cells, VGSC activity increases invasiveness by stimulating cysteine cathepsin activity (Gillet et al., 2009). Non-conductive roles of VGSCs, with possible oncological relevance, such as direct involvement in cell adhesion, are also emerging (Chioni et al., 2009; Nelson et al., 2014). In general, VGSCs appear to be associated with the regulation of some of the processes implicated in the metastatic cascade; for a recent review, see Fraser et al. (2014). Preclinical studies have shown that targeting VGSC inhibits breast tumor growth and metastasis formation (Nelson et al., 2015).

Another interesting perspective is the implication in cancer of neurotransmitter-gated ion channels, which are becoming increasingly recognized as widely expressed outside the nervous system (Becchetti, 2011; Al-Wadei et al., 2012). Most evidence concerns the nicotinic acetylcholine receptors (Egleton et al., 2008; Schuller, 2009; Ambrosi and Becchetti, 2013), which have been shown to regulate cell proliferation, apoptosis and angiogenesis in several tumors, including lung cancers (Dasgupta et al., 2006; Lam et al., 2007; Egleton et al., 2008). Genome wide association studies have implicated variants in chromosome 15q24-25 region in the development of lung cancer and nicotine dependence (Amos et al., 2008; Hung et al., 2008; Thorgeirsson et al., 2008). This locus contains the *CHRNA3/A5/B4* gene cluster, which encodes for α3, α5 and β4 nAChR subunits. Silencing these genes as well as inhibiting the

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