TARGETING THE ETHER-À-GO-GO ION CHANNELS IN CANCER THERAPY: CURRENT KNOWLEDGE AND FUTURE PERSPECTIVES

DANIEL ȘTERBULEAC[1*], DUMITRU COJOCARU[2,3]

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Abstract: Members of the Ether-à-go-go (Eag) group of potassium channels, including the human Ether-à-go-go (hEag) and the human Ether-à-go-go-related gene (hERG) ion channels, have been shown to play important roles in cancer pathogenesis and to regulate many aspects of tumour development. It was shown that they are frequently overexpressed or ectopically expressed in different human cancers, which, combined with their cell surface expression, led to different attempts at finding their therapeutic potential as promising cancer therapy targets. This review summarizes the current knowledge drawn from the results of independent studies performed to identify sought-after alternatives of targeting these ion channels in cancer therapy.

INTRODUCTION

Ion channels are transmembrane proteins that allow the passive passage of specific ions through the plasma membrane. Their cell surface expression is correlated with a high therapeutic potential, since many drugs targeting them may not need to enter into cells to exert their beneficial effects. Such therapeutic effects usually consist either in blocking the ion conduction or oppositely, to activate the channel's function and sustain an overly increased flow through them. Although ion channels form a large pharmacological target group, no ion channel is currently targeted in antitumour therapy, despite recent intense efforts directed towards deciphering their role, expression and therapeutic potential, which lead to an impressive accumulation of experimental data (Becchetti et al, 2013).

There are many identified ion channels which play roles in the progression of cancer, but evidence accumulates in favor of potassium (K^+) channels, which are frequently overexpressed in tumour cells and have been demonstrated to play roles in basically all cancer cell-specific physiological processes (Huang and Jan, 2014). A particular group in the K^+ channels superfamily is named Ether-à-go-go, or Eag. In humans, this group is formed by the Ether-à-go-go (hEag), Ether-à-go-go-related gene (hERG) and Ether-à-go-go-like (hELK) ion channels. They all share several structural features, but have different physiological parameters. Each one of the channels may be coded by different genes (for example, hEag1 is coded by *KCNH1* while hEag2 is coded by *KCNH5*) and may present different isoforms after differential splicing. All channels have various, but different, functions in the human body. The hEag channel is expressed in fusing myoblasts, the retina and the central nervous system. The hERG channels are expressed in a wide variety of tissues, being critically responsible for conducting the rapid depolarizing current which follows the cardiac action potential, which is why several non-specific hERG1 blockers have been withdrawn from the market, as the channel became an important pharmaceutical antitarget (Raschi et al, 2008).

The Eag channels have important roles in cancer cells of various origins (Tang et al, 2016). They have been shown to possess oncogenic properties when their expression is enlarged and are also frequently expressed or overexpressed in tumour cells (Camacho, 2006; Hemmerlein et al, 2006). A large amount of evidence emerged, all of which show how hEag and hERG channels interact and regulate specific cancer cells functions. In fact, hEag has a limited tissue distribution, but combined with its ectopic expression in cancer makes it a highly suitable biomarker and potential antitumour target, unlike hERG, which is expressed more ubiquitously and its blockade has serious cardiac side effects. The channels play important roles in cancer cells and regulate several aspects of cancer pathogenesis and progression (Arcangeli and Becchetti, 2015). Here, we present several lines of evidence that emerged during recent years regarding the potential of Eag channels as promising anticancer targets and the various strategies that were demonstrated to have limited experimental benefits. Then, we present several perspectives, which could well be considered to be further employed in order to draw new information about these channels and to ultimately target them in cancer therapy.

TARGETING EAG CHANNELS WITH SMALL MOLECULES

Targeting ion channels by small molecules is currently used in treating a variety of conditions, due to the accessible location of ion channels (Bagal et al, 2013). An impressive number of compounds have been found to inhibit the hERG ion channel, due to its unique large cavity which can accommodate many types of blockers. Interestingly, most known blockers of hERG and hEag, like imipramine or astemizole, have been shown to inhibit cell proliferation in some cancer cell lines by targeting hERG (García-Ferreiro et al, 2004). However, most hERG blockers are also blockers of hEag, therefore it was assumed that the anticancer effects could be due to blocking both K^+ currents at once. Nevertheless, hERG blockers usually have a lower affinity for the hEAG channels, which is usually attributed to the lack of inactivation of the latter. Inactivation of hERG is thought to favor a much better placement of important residues in the lower cavity, to contact the blocking compounds (Perry et al, 2010), even though there are some identified exceptions to this general rule, (see below). In fact, to date, it was difficult to make any correct interpretation of the role of hEag1, since there is no specific potent hEag1 blocker. Such a blocker would have important consequences, as it should have important antitumour properties, given the fact that specific hEag1 antibodies have been shown to significantly decrease proliferation of cancer cells (explained in another part of the manuscript). An interesting challenge could be finding through drug design processes or high-throughput screening certain compounds which could bind differently to the ion channels' inner cavity, thus having an increased blocking effect on hEag1 and not on the related hERG channels. Such a hypothesis was recently launched as it is believed that clofilium, another non-specific blocker, could be chemically modified to specifically target hEag1, given its particular blocking determinants (Șterbuleac and Maniu, 2016). Particular structural features of the two channels (on the intracellular activation gate), combined with a new binding mode of clofilium to the hERG channel, would mean that increasing the polarity of the molecule near the phenyl group should generate the needed larger affinity for the hEAG1 channel (Șterbuleac and Maniu, 2018). Since hERG blockers exert different types of side effects, not all of which are very dangerous, it was also hypothesized that they could be repurposed for cancer therapy under careful monitoring (Huang and Jan, 2014).

Another promising potential was brought up, regarding the involvement of hERG channels in leukemias. The hERG-encoding gene, *KCNH2*, expresses at least two different hERG isoforms, A and B, corresponding to two different ion channels, hERG1A and hERG1B (Gasparoli et al, 2015). It was noticed that hERG1A is the dominant form expressed in myocytes, but hERG1B is frequently overexpressed in leukemias. A promising drug candidate has been identified, which is able to block the hERG1B ion channel without interfering with hERG1A function, named CD-160130. It was also shown that this drug has important *in vitro* and *in vivo* antileukemic effects, thus opening the door to subsequent research in this promising alternative anticancer combination between target and targeting compound.

TARGETING EAG CHANNELS WITH ANTIBODIES

Employing antibody therapy in cancer therapeutics is being given full consideration, due to recent advances in this research field (Wold et al, 2016), but significant progress is still awaiting in order to use this approach to block ion channels function and treat human diseases (Sun and Li, 2013). In order to identify novel therapeutic ion channels antibodies, several issues have to be addressed (Wilkinson et al, 2015). In fact, the first monoclonal antibody that specifically

inhibited only one K^+ channel from this group, hEag1, showed that specific targeting of this channel has important antitumour activity (Gómez-Varela et al, 2007). This antibody had antigrowth effect *in vivo* and on several cell lines expressing hEag1. As such, it showed that sole blockade of hEag1 is sufficient for good anticancer effects. However, as far as we know, no other similar studies were designed.

TARGETING GENE EXPRESSION BY siRNA

Small-interfering RNA (siRNA) therapy is a promising new field of research and relies on using specific RNA molecules which should combine with cellular RNA transcripts and silence the respective gene (Gavrilov and Saltzman, 2012). Nevertheless, it faces serious challenges, probably the most important one relying on the challenges to design carriers to deliver siRNA to target specific cells. This method consists in applying small RNA molecules complementary to the mRNA, therefore silencing specific gene expressions. In line with other studies previously presented, silencing the Eag channels' gene expression also revealed antitumour properties, but these studies were performed only using cancer cell cultures. For example, hEag1 siRNA significantly reduced proliferation and colony formation (Wu et al, 2015; Weber et al, 2006). Little to no effect was observed on apoptosis, suggesting that the effect is cell cycle-specific, but significant beneficial effects were observed on cell adhesion and migration of osteosarcoma cell lines. A similar study was performed on hERG (Zeng et al, 2016) and showed comparable results, although in this case siRNA induced apoptosis. These studies are important since they brought additional information regarding the complex cellular signaling processes in which Eag channels are involved and include STAT3, VEGF or NF-κB, proteins known to be involved in cancer progression. Interestingly, different cellular signaling pathways were activated by interfering with each one of the channels.

DUAL-TARGETING OF EAG CHANNELS

As presented so far, it can be noticed that the studies performed on the anticancer therapeutic potential of these channels either need further extension or show potential limitations. However, several lines of evidence exist, showing that using multiple types of channel targeting has combined effects. It was demonstrated that a hEag1 specific antibody coupled to the soluble tumour necrosis factor-related apoptosis-inducing ligand (sTRAIL), which is a known promising anticancer candidate, led to an improved cancer cell-targeting on different cancer cell lines than application of antibody alone (Hartung et al, 2011). Using calcitriol (which blocks hEag1 while having other beneficial effects) and astemizole combined showed improved *in vivo* effects than application of either one alone, which also attests that more efficient results can be seen by using a combined targeting strategy (García-Quiroz et al, 2014).

PERSPECTIVES

As mentioned, there are several lines of research that merit full consideration towards finding novel anticancer therapeutics or to repurpose other drugs. Clofilium is an interesting candidate to be chemically altered and to identify the first hEag specific blockers. Recent structures of hERG1 and hEag1 were identified and could be used to aid the drug design processes. Then, it would be interesting to see if such blockers could be optimized for lesser toxicity and would have anticancer effects. Indeed, an alternative approach is a comprehensive screening of a large compound library, in order to identify specific potent hEag1 blockers or chemical features of

such a blocker. Such a study is much sought-after and would bring important information and knowledge in this field.

Other pieces of information are still missing. An integration of the two channels in the complex signaling pathways of normal and cancer cells would be quite challenging, but it should rely on a comprehensive biochemical and cellular experimental perspective. It should also be based on deciphering the precise biogenesis and trafficking of the two channels, which could then be integrated altogether in the complex so-far-known architecture of the cellular machinery. Such machinery is highly unregulated during cancer, which also leads to a genomic instability, which then influences, through mutations, the structure and function of ion channels. It has to be analyzed whether disfunction of ion channels occurs as a consequence of genome instability and if this can be reversed.

Antibody therapeutics is another promising line that could bring new evidence about the role of Eag channels in cancer cells. Yet, targeting ion channels in cancer with antibodies faces serious challenges and the difficulties regarding it might also indicate the scarcity of studies in this specific field. Nevertheless, given the highly relevance of the Eag channels in cancer pathogenesis and the recent evidence, studies should rely on deciphering whether multiple ion channels targeting, used in conjunction with other types of anticancer targeting, would bring improvements. Interesting results were obtained by combining multiple types of targeting, even if such studies were limited to cancer cell cultures. This merits further studies to be also used in a clinical basis, thus avoiding hERG-related side effects while personalizing the treatment independently for each patient. Targeting Eag could well serve as a means of improving the overall classical chemotherapeutic treatment, by integrating Eag blockers in the treatment scheme while taking account of patient's genetic profile or compound metabolism. This is why the involvement of Eag channels should also be highlighted using pharmacogenetics approaches, which will definitely yield important cellular interactions specific to these channels and that may be further employed in other studies.

CONCLUSIONS

The Eag channels have important roles in cells and their function is linked to various physiological processes. Despite their relatedness, the hERG and hEag play significantly different roles in various cell types. It is acknowledged that Eag channels play significant roles in cancer progression and their targeting in anticancer therapy is one of the most promising research premises in this research area. Several studies showed that interfering with the channels' function leads to anticancer effects. All of the presented strategies merit further development in order to relate Eag channels to specific roles played in cancer and to identify clinically-relevant active molecules to target Eag channels in cancer therapy.

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[1]Doctoral School of Biology, Faculty of Biology, "Alexandru Ioan Cuza" University of Iasi, 20A Carol I Blvd., 700505, Iasi, Romania
[2]Laboratory of Biochemistry and Molecular Biology, Faculty of Biology, Department of Biology, "Alexandru Ioan Cuza" University of Iasi, 20A Carol I Blvd., 700505, Iasi, Romania
[3]Academy of Romanian Scientists, 54 Splaiul Independenței, 050094, Bucharest, Romania
daniel.sterbuleac@gmail.com