Eag1: An Emerging Oncological Target

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Abstract

Emerging evidence indicates that ion channels act in a variety of physiologic and pathologic processes beyond electronic signal transmission, including in cancer. We recently found that the potassium channel Eag1 can mediate cancer progression and that a monoclonal antibody, which inhibits Eag1 action, can effectively restrict cancer cell proliferation. We discuss how Eag1 targeting may be useful in diagnostic or therapeutic settings. [Cancer Res 2008;68(6):1611–3]

An Emerging Class of Cancer Targets

Ion channels constitute a numerous, abundant, and well-characterized group of proteins. Their therapeutic value derives from their extracellular accessibility and because their functional behavior can be studied at the single molecule level in real time, allowing detailed optimization of drugs. This particular advantage was not routinely applied to screening programs in the pharmaceutical industry because of limited throughput. Isotope fluxes and fluorescent indicators were used instead. The introduction of novel mid-throughput electrophysiologic techniques has now increased the interest in this class of molecules. Nevertheless, ion channel modulators have been used for a long time in the management of, for example, hypertension, diabetes, and pain.

Although well established as therapeutic targets in excitable cells, the role of voltage-gated potassium channels in the physiology of nonexcitable cells has only recently caught the attention of the scientific community. Oncology is no exception, and although hints for the implication of ion channels in the pathogenesis and evolution of cancer have been available for at least 20 years (1), only recently a significant number of channels have been directly linked to cancer, including sodium, chloride, calcium, and potassium channels (e.g., refs. 2–5).

There are many analogies between ion channels and other membrane receptors from the point of view of drug design. On a G protein–coupled receptor or an enzymatic receptor, it is possible to interfere from the extracellular side with the binding of the ligand to its receptor, either directly or allosterically. All other possibilities require access to the intracellular milieu. Ion channels are different in the sense that their primary function, ion permeation, requires physical contact between intracellular and extracellular media and are therefore tractable in intact cells without needing to cross the plasma membrane. Moreover, the interference with ion permeation can be rather selective because the physical interaction with the channel does not occur in the permeation pathway itself (which is very similar in all channels with a given ionic selectivity) but at the mouth of the channel, which has a variable sequence among the various channels.

Inhibition of Eag1 by a Monoclonal Antibody

Among all voltage-gated potassium channels, two members of the ether-á-go-go family, HERG (KCNH2, Kv11.1; ref. 5) and Eag1 (KCNH1, Kv10.1; ref. 3), have extensively been studied about their implication in oncology. We have used Eag1 as a model for several reasons. First, Eag1 is practically not detected in normal tissues outside the central nervous system, but is aberrantly expressed with very high frequency (>75%) in tumor cells from diverse origin (6, 7). Channel activity has been measured in tumor cells (8–11), showing that that the channel is located at the cell surface and allowing the use of Eag1 to identify tumor cells. Second, it has been shown that the inhibition of the channel expression and/or function reduces tumor progression (10, 12, 13). An obvious conclusion of these functional features of Eag1 is that specific blockade of the channel with a compound effectively excluded by the blood brain barrier could lead to a selective inhibition of the proliferation of tumor cells. A large number of compounds have been described to block Eag1, but none of them is specific. All known compounds able to inhibit Eag1 also block HERG, implicating a cardiac safety problem (14) because they could induce arrhythmia through prolongation of the QT interval. Although this does not totally disqualify Eag1 as a target for small-molecule inhibitors, it prompts the design of alternative strategies.

We therefore approached the design of an anti-Eag1 functional antibody (15). An antibody able to inhibit channel function would offer several advantages. On the one hand, it should not recognize related ion channels, and it should bind only to cells expressing the epitope on their surface, which is essentially to tumor cells in the case of Eag1. Previous attempts to generate functional antibodies against ion channels gave rise to polyclonal sera (16), but there were no precedents of monoclonal antibodies that effectively block channels in intact cells. For Eag1 (and most other ion channels), the domains exposed to the extracellular milieu represent a small fraction of the total protein, limiting the areas susceptible to be used as antigen. The problem was addressed by constructing a fusion protein using two segments of the Eag1 channel: a region between the fifth transmembrane segment and the pore-lining region (the E3 region; ref. 16) and a segment in the COOH terminus that we had previously shown to form a very stable tetrameric coiled-coil (17). This fusion protein was used as an antigen and gave rise to a set of antibodies that were subsequently selected for specificity and functional inhibition of the current by electrophysiologic techniques.

We could show that the selected antibody can efficiently reduce ion flow through Eag1 channels in intact cells. In addition, it does not bind to Eag2 (KCNH5, Kv10.2) or to the physiologically relevant HERG1, which is involved in controlling the length of the QT interval in ventricular repolarization. This antibody is therefore the first selective blocker of Eag1. Epitope mapping revealed that the sequence recognized by the antibody lies close to the pore-lining residues, in an area with very low homology to other channels. Further characterization will be required to determine the exact mechanism of action of the antibody in terms of channel blockade.
It is not clear whether the antibody inhibits ion permeation directly or if it inhibits gating through allosteric interaction, although the fact that opening of the channel is required for the block to be established is suggestive of an open channel block mechanism. The availability of the antibody allowed testing if block of Eag1 reduced tumor cell growth. Thus far, all reports on the involvement of ion channels in proliferation were based either on the specific inhibition of the expression of an ion channel or on the use of relatively
unselective blockers. Toxins, which are also as antibodies, have not been shown to inhibit tumor cell proliferation, although there are toxins against tumor-relevant channels (e.g., ref. 18). We used our specific blocking antibody to show that inhibition of the function of a particular ion channel, in this case Eag1, is able to reduce colony formation of several tumor cell lines in a dose-dependent manner. An isotype antibody as well as an Eag1-specific antibody that did not block ion flow through Eag1 did not affect colony formation efficiency, strongly indicating that the functional activity of Eag1 is required for it to favor malignant phenotypic features such as substrate-independent proliferation. In vivo i.p. administration of the antibody was able to reduce tumor growth in two different models, although the inhibition was modest and did not happen in all cell types tested. It might be important to note that these experiments were carried out in immune-depressed mice, which are incompetent for antibody-induced immune responses. No toxic effects or behavioral changes were observed at the doses tested. Although it is possible that an antibody actively crosses the blood–brain barrier (reviewed in ref. 19), no significant neurotoxicity was expected in immunodepleted mice because effective blockers of Eag1 that readily reach the brain parenchyma (e.g., imipramine) do not exhibit massive side effects.

Earlier, we have summarized some of the features that make ion channels especially attractive for drug targeting. The experiments described here are a proof of principle for the use of ion-channel specific antibodies as diagnostic and therapeutic instruments. We have narrowed down the region within the E3 loop of ion channels where specific antibodies can bind and inhibit channel function. Interestingly, other antibodies binding slightly upstream in the channel sequence were not functional. Because the sequence similarities between the various ion channels in this area are low, it should be possible to design specific antibodies against particular channels. These can be used in specifically determining the roles of each channel subtype, both in physiology and pathophysiology.

More interestingly, functional antibodies can be used to validate Eag1 as a cancer target as in the example described above. Unpublished experiments from our laboratory indicate that Eag1 expression is usually not a triggering event in the process of malignant transformation, but rather occurs later in the development of the tumor, because robust expression can be detected in animal models in which carcinogenesis is initiated by well-defined genetic causes. Nevertheless, Eag1 has the added advantages of having an extraordinarily low background expression and being very frequently detected in solid tumors. Although there is no evidence of surface expression of the channel in actual human clinical samples in situ, tumor models reveal that anti-Eag1 antibodies can bind to xenografted tumors in alive animals, as shown by specific accumulation of fluorescently labeled antibody in the tumor (20). Additionally, the antibody could be used as a carrier for radionuclides or toxic molecules because it has proven efficacy for other molecules. In this context, it should be possible to design a molecule able to selectively target tumor cells and attack them on several different fronts, functionally inhibiting Eag1 while also delivering cytotoxic drugs (Fig. 1).

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References

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