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Minireview

Potassium channels as tumour markers

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Abstract An increasing number of ion channels are being found to be causally involved in diseases, giving rise to the new field of "channelopathies". Cancer is no exception, and several ion channels have been linked to tumour progression. Among them is the potassium channel EAG (Ether-a-go-go). Over 75% of tumours have been tested positive using a monoclonal antibody specific for EAG, while inhibition of this channel decreased the proliferation of EAG expressing cells. The inhibition of EAG is accomplished using RNA interference, functional anti-EAG1 antibodies, or (unspecific) EAG channel blockers. Fluorescently labelled recombinant Fab fragments recognizing EAG allow the distribution of EAG to be visualized in an in vivo mouse tumour model.

Keywords: EAG; Cancer; Ion channels; Oncogene; Near-infrared imaging

1. Historical overview

A growing number of ion channels are being implicated in tumor progression, with sodium and potassium channels among them. With respect to potassium channels, it has been postulated that they are important for proliferation since they influence cell volume [1]. However, this would be a general mechanism that applies to all types of potassium channels, and this is not the case [2]. To date, over 70 types of potassium channels are known [3], but only a few have been directly implicated in cell proliferation and tumour growth. The best studied cases include Kv1.3 (*KCNA3*) [4–8], IKCa1 ($K_{Ca}3.1$, *KCNN4*) [9], TASK-3 ($K_{2P}9.1$, *KCNK9*) [10], HERG (Kv11.1, *KCNH2*) [11–15] and EAG1 (KV10.1, *KCNH1*) [16,18]. Taking EAG as an example, what is the evidence for its involvement in cell proliferation and tumour progression?

2. EAG is involved in cell cycle and proliferation

Initially, it was observed that the activation kinetics of EAG markedly depend on the holding potential, becoming more rapid at depolarized potentials [19], and that the *Drosophila* variant is permeable to potassium and calcium, as well as being modulated by cAMP [20]. In addition, extracellular magnesium concentrations are able to modulate the kinetics [21]. The first indications that EAG is involved in the cell cycle came from the observation that mitosis-promoting factor affects the amount of current through this channel [22] and that the particular cell-cycle stage modulates its selectivity to caesium and block by intracellular sodium [23]. Furthermore, the microtubule depolymerization that occurs during mitosis produces similar effects [24], further strengthening the evidence that EAG is modulated during cell cycle.

Similarly to most potassium channels, EAG subunits form a functional channel when assembled into tetramers. Given that there is another human isoform of EAG, EAG2, heteromultimeric channels comprised of EAG1 and EAG2 can be formed. The specificity of this interaction is determined by the C-terminally located tetramerizing coiled–coiled (TCC) domain which also determines the possible tetramer composition, as it for example precludes the formation of functional channels composed of EAG and HERG subunits [25]. During these studies, it became evident through electrophysiological measurements that few functional EAG channels exist despite the fact that significant intracellular levels of EAG protein could be detected. This discrepancy is probably caused by the retention of EAG in the ER (which would be consistent with the fact that the EAG sequence contains an ER-retention signal LRKR [26]), and modulation of its trafficking by glycosylation [27].

3. Inhibition of currents through EAG

Outside the CNS, EAG is a specific marker for tumor tissue [16–18] and its inhibition by astemizole or the tricyclic antidepressant imipramine reduces the proliferation of tumor cells [28,29]. Obviously, an EAG inhibitor would be a helpful tool for the development of diagnostic and therapeutic agents and therefore the blocking mechanism of EAG1 channels by astemizole and imipramine was investigated in detail [30]. Unfortunately, these substances are unspecific and also affect the human EAG-related gene product, HERG.

Another strategy is the development of specific antibodies that recognize the extracellularly accessible tumor markers. The efficiency and convenience of the use of antibodies against tumor-specific markers, such as HER-2/neu, are sometimes combined with other chemotherapeutics to increase response rates or reduce the effective dose [31]. Recent advances in near-infrared fluorescent (NIF) imaging [32], in combination with specific

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antibodies, have allowed in vivo molecular imaging of small animals. For this approach, the antibodies need to be labeled with infrared emitting dyes such as AlexaFluor 680 or Cy5.5.

We tested this approach by labeling a monoclonal antibody specific for EAG1 with a secondary antibody labeled with AlexaFluor 680 [33]. The resulting complex was injected into immuno-suppressed mice grafted with MBA-MB-413S human mammary carcinoma cells (which are positive for EAG1 expression) as a tumor model. Twenty-four hours after injection of 100 μg of the anti-EAG1–Alexafluor complex, the primary tumor as well as the corresponding sentinel node, was clearly visible (see Fig. 1). This indicates the association of this NIF complex with the extracellular EAG1 epitope [33].

4. Fluorescently labeled single chain anti-EAG antibody

A disadvantage of this approach is the large size of the fluorescent complex that consists of two complete antibodies and the conjugated fluorescent dye. Direct labeling of the antibody with the fluorescent dyes caused a significant reduction in affinity, possibly due to their conjugation to several lysine residues present in the antibody's complementarity determining regions (CDR's). We have addressed this issue by generating a recombinant single-chain anti-EAG1 antibody comprising only the Fab' domain, which did not significantly differ in either affinity or specificity to the whole antibody, and to which a poly-lysine chain was linked, thereby increasing the labeling efficiency. In this way, a much smaller anti-EAG1–Cy5.5 complex was generated, which can more easily penetrate dense tumour tissue. In addition, it is also now possible to produce an engineered protein consisting of the single-chain antibody and a modified fluorescent protein with red-shifted spectrum.

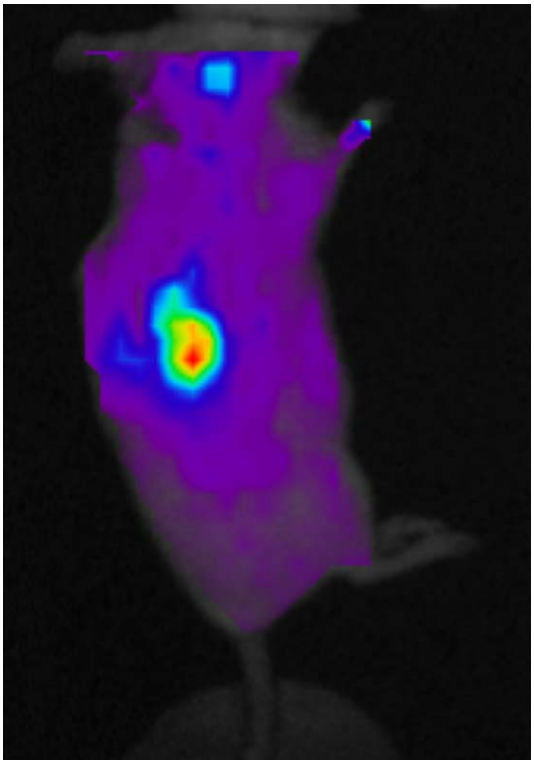

Fig. 1. False-color image of NIR intensities from Cy5.5 conjugated to anti-EAG Fab fragment 24 h after i.v. injection of the conjugate. The mouse had been grafted with MBA-MB-435 cells and a palpable tumour had just developed. A shift towards the red indicates higher intensities. The lifetime of the fluorescence observed corresponds to Cy5.5.

5. In vivo imaging of EAG

Fig. 1 shows an image of a mouse 24 h after i.v. injection of a poly-lysine containing recombinant anti-EAG1-Fab' fragment I conjugated to Cy5.5. The immunodeficient mouse had previously been grafted with MBA-MB-435S cells. The false colors correspond to NIR intensities obtained using an eXplore Optix System (General Electric Healthcare, London, Canada). The mouse was anaesthetized by inhalation of isofluorane, a procedure that can be applied repeatedly without harming the living animal.

In summary, EAG is a novel and highly significant tumour marker that has diagnostic and therapeutic potential. Being a potassium channel, it is extracellularly accessible and can be molecularly labelled in vivo using NIR imaging.

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