REVIEW-SYMPOSIUM

The role of potassium channels in tumours of the gastrointestinal tract: a focus on the human ether-à-go-go related gene 1 channels

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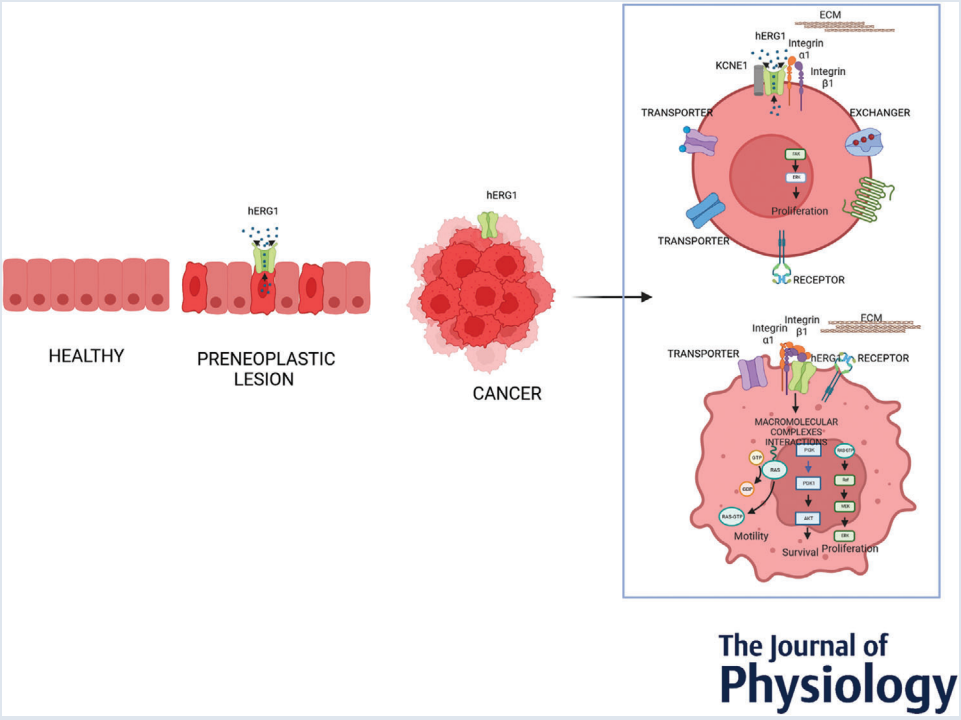

Abstract Potassium channels are often dysregulated in tumours of the gastrointestinal (GI) tract. Among them, the voltage-dependent channel $K_V11.1$, also known as human ether-à-go-go related gene 1 (hERG1), is frequently expressed in tumours and precancerous lesions of the GI tract. In precancerous lesions, hERG1 behaves as a progression factor, contributing to identifying those patients whose lesions can progress towards true cancers. In advanced cancers, such as colorectal and pancreatic cancer, a high hERG1 expression represents a negative prognostic factor, contributing to identifying high risk patients. The only exception is represented by neuroendocrine cancers of both the ileum and the pancreas, where hERG1 represents a positive prognostic factor for survival. In GI tumours, hERG1 can function either as a true channel, allowing outward potassium ion flux and membrane repolarisation, or in a non-canonical, non-conductive way. This occurs because, in cancer, hERG1 forms complexes with different

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plasma membrane and cytosolic proteins, instead of classical accessory subunits. In particular, hERG1 forms a complex with the β1 subunit of integrin receptors: the hERG1–β1 complex. Growth and chemokine receptors, small GTPases, phosphoinositide 3-kinase, as well as other ion transporters or channels, are also recruited in the hERG1–β1 complex. The formation of multiprotein channel complexes represents an emerging mechanism allowing functional channel networking in both excitable and non-excitable cells. hERG1 represents a prototype of how multiprotein complexes operate in tumours, that is, giving rise to signalling hubs which can transmit and modulate signals arising from the tumour microenvironment, hence contributing to tumour progression and malignancy.

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Abstract figure legend The hERG1 channel is not expressed in healthy epithelial tissue while in preneoplastic lesions of the GI tract it might be expressed to a different extent in altered cells (indicated in red). In some of these cells the channel might be expressed retaining at least in part its correct conductive properties (green channel with potassium ion flux, represented by the blue dots). In cancer cells the hERG1 channel might be present with altered function. From a molecular point of view, a normal cell (upper right) is usually characterised by the presence of hERG1 coupled with its accessory subunit KCNE1, β1 integrin and other ion channels, transporters and receptors. On the contrary, in a cancer cell (lower right) hERG1–β1 forms complexes in which other molecules, such as transporters and receptors are involved and sustain downstream signalling, regulating proliferation, motility and survival. Created with BioRender.com.

Introduction

By controlling electrical and ionic homeostasis at the membrane level, ion channels regulate different aspects of cell physiology. Furthermore, ion channel dysfunction is relevant in different pathologies, including cancer (Prevarskaya et al., 2018). Indeed, ion channels are frequently over- and misexpressed in tumours and their activity can contribute to regulating different cancer hallmarks (e.g. cell proliferation, survival and apoptosis, migration, invasion, and angiogenesis). In this context, ion channels can be considered novel tumour biomarkers (Lastraioli, Iorio et al., 2015), to be exploited for diagnostic and therapeutic purposes (Arcangeli et al., 2009; Marshall & Djamgoz, 2018). Among ion channels which are frequently dysregulated

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in tumours, K^+ channels are pivotal, being broadly expressed in several types of tumours, either primary samples or cells (Huang & Jan, 2014; Pardo & Stühmer, 2014), where they collectively modulate the cancerogenic processes (Arcangeli & Yuan, 2011; Arcangeli et al., 2009; Becchetti, 2011).

How do K^+ channels work in cancer to regulate the cancer hallmarks? The K^+ efflux provided by K^+ channels can modulate cancer cell behaviour by (1) providing the electrochemical force needed for the influx of Ca^{2+} (e.g. through store-operated Ca^{2+} channels), which is known to be important for G_0/G_1 and G_1/S transitions, (2) by transiently hyperpolarising the membrane potential, which is also an important feature for cell cycle progression, or (3) regulating cell volume. Furthermore, depolarisation and Ca^{2+} regulate exocytosis of hormones and paracrine factors (growth factors, cytokines, etc.), which in turn control different cancer-related mechanisms. Finally, K^+ channels can have non-canonical roles. In other words, they may also work in a non-conductive manner by promoting signal transduction pathways involved in cell proliferation, survival, motility, etc., through interaction with other membrane proteins such as integrins, growth factors receptors (GF-R), chemokine receptors (Ck-R), etc. (Becchetti et al., 2019, 2022). These aspects will be particularly addressed in the present review.

K^+ channels expression and role in cancer of the gastrointestinal tract

The gastrointestinal (GI) tract is the anatomical site of some of the most frequent and deadliest human cancers. According to Globocan (i.e. the Global Cancer Observatory, belonging to the International Agency For Cancer Research) 2020 estimates (Globocan 2020, https://gco.iarc.fr, accessed on 29 June 2022), GI tract tumours still represent a major health problem since they all belong to the top 10 most lethal cancers. Indeed, mortality rates for colorectal cancers (CRC) are located in the second position, while gastric cancer (GC) and pancreatic cancer (PC) are the fourth and seventh leading cause of mortality from cancer worldwide, respectively. In addition, a sharp increase in incidence and mortality for oesophageal cancer (OeC), in particular of the adenocarcinoma histological subtype (OeAC), has been observed in the last decades, so that OeC now ranks sixth. GI cancer are generally more frequent in developed countries (Globocan 2020, https://gco.iarc.fr, accessed on 15 September 2022) due to well-known risk factors such as diet and physical activity, among others. Nevertheless, differences are also present among the different GI cancers (Globocan 2020): for instance, CRC and PC are more frequent in Europe, North America and Oceania with respect to other countries, while GC and OeC have the highest incidence in Asia. Also, sex-related factors play a role in CRC, PC, GC and OeC, being more frequent in males.

Like other cancers, GI tumours can be preceded by precancerous lesions, whose onset and evolution must be carefully monitored since they are likely to evolve into true malignant tumours. Examples are Barrett's oesophagus (BOe), a precancerous lesion for OeAC, gastric dysplasia, which often progresses into GC, colon rectal adenomas, which often precede CRC, as well as the hyperplastic and dysplastic lesions of the pancreatic ducts called pancreatic intraepithelial neoplasia (PanIN), which are considered precancerous lesions for PC.

Potassium ion channels are present in tumours and precancerous lesions of the GI tract, as extensively reviewed by different groups (Anderson et al., 2019; Hofschröer et al., 2021; Lastraioli, Iorio et al., 2015). For the purposes of the present review, we will focus only on some selected K^+ ion channels. The Ca^{2+}-dependent K^+ channel $K_{Ca}3.1$ (encoded by the *KCNN4* gene) is highly expressed in CRC human primary samples and CRC cells. In particular, cisplatin-resistant CRC cells expressed higher levels of $K_{Ca}3.1$ channels, compared with cisplatin-sensitive CRC cells (Pillozzi et al., 2018). In resistant cells, $K_{Ca}3.1$ activators (e.g. SKA-31) had a synergistic action with cisplatin in triggering apoptosis and inhibiting proliferation (Pillozzi et al., 2018). Cisplatin uptake into resistant cells depended on $K_{Ca}3.1$ channel activity, as it was potentiated by $K_{Ca}3.1$ activators. Similar results were produced by riluzole, which is able to both activate $K_{Ca}3.1$ and inhibit hERG1 channels (see below). Indeed, an interesting cross talk between the two channels was reported, since $K_v11.1$ blockade led to increased $K_{Ca}3.1$ expression and thereby stimulated cisplatin uptake, and the combined administration of a $K_{Ca}3.1$ activator and a hERG1 inhibitor also overcame cisplatin resistance on a CRC mouse model (Pillozzi et al., 2018). The clinical relevance of $K_{Ca}3.1$ expression in CRC was hypothesised by Lai et al. (2013), although not validated afterwards in a larger patient cohort of CRC samples belonging to different TNM stages (Muratori et al., 2016). $K_{Ca}3.1$ channels are also upregulated in PC, and their overexpression is associated with poor patient survival (Hofschröer et al., 2021). In PC, $K_{Ca}3.1$ channels regulate cell proliferation, cell migration and invasion (Bonito et al., 2016; Schwab et al., 2012). The voltage dependent K^+ (K_V) channel K_V 7.1, commonly known as KCNQ1 (encoded by the *KCNQ1* gene), is also relevant in GI tumours. KCNQ1 is physiologically expressed in the heart (Barhanin et al., 1996; Sanguinetti et al., 1996), as well as in some epithelia, including gastric and intestinal (Vallon et al., 2005). Thanks to the association with different accessory subunits (KCNE2 and KCNE3), KCNQ1 regulates different cellular activities (Anderson

et al., 2019). Based on numerous genetic, biomolecular and immunohistochemistry data, KCNQ1 apparently functions as a tumour suppressor in GI cancers (Morris et al., 2017). Although the mechanism underlying tumour suppression is not well understood, it is worth noting that KCNQ1 has been implicated in the Wnt/β-catenin pathway (Rapetti-Mauss et al., 2020), which is frequently deregulated in GI cancers, in particular in CRC. Interestingly, KCNQ1 and β-catenin physically interact in GI tumours forming a molecular complex whose functional relevance in GI tumours is discussed below.

The EAG family of voltage-gated K^+ channels, whose most important members are K_V10.1, also known as EAG1, encoded by the *KCNH1* gene, and K_V 11.1, also known as hERG1, encoded by the *KCNH2* gene, is highly represented in human cancers and deeply involved in the regulation of different cancer hallmarks (Pardo & Stühmer, 2014). In the healthy organism, EAG1 and hERG1 are expressed in excitable cells such as neurons and muscle cells (Cázares-Ordoñez & Pardo, 2017; Ouadid-Ahidouch et al., 2016; Sanguinetti et al., 1995). In particular, hERG1 channels are key determinants of the potassium current (I_{Kr}) responsible of the fast-repolarising phase following the cardiac action potential (Sanguinetti et al., 1995). Subjects carrying germline mutations of the *KCNH2* gene develop the so called by type 2 long QT syndrome, a life-threatening ventricular arrhythmia (Vandenberg et al., 2012). Both channels have been detected in many other tumour cell lines and primary tumours (Arcangeli et al., 2009; Lastraioli, Iorio et al., 2015; Pardo & Stühmer, 2014). In GI tract tumours, the *KCNH1* transcript is expressed in oesophageal squamous cell carcinoma (OeSCC), where it represents an independent negative prognostic factor (Ding, Wang et al., 2008). The *KCNH1* transcript is also expressed in primary human CRC samples, along with other K^+ channel encoding genes, and genomic amplification of the *KCNH1* gene is an independent marker of adverse prognosis (Ousingsawat et al., 2007). Finally, EAG1 is expressed in PC, particularly pancreatic ductal adenocarcinoma (PDAC), cells where it can represent a targetable biomarker (reviewed in Pardo & Stühmer, 2014). The case of hERG1 and its roles in GI tumours is addressed in more detail below.

The expression of hERG1 in GI tumours and precancerous lesions

While hERG1 is not expressed in normal non-excitable tissues (Becchetti et al., 2022; Duranti & Arcangeli, 2019), it is frequently upregulated in several types of cancers, including those of the GI tract. In the oesophagus, The *KCNH2* gene and the corresponding protein are expressed in a high percentage of oesophageal squamous cell carcinoma (OeSCC) (Ding, Wang et al., 2008) as well as in oesophageal adenocarcinoma (OeA) (Lastraioli et al., 2006), while expression is absent in healthy oesophageal squamous epithelium (Ding, Luo et al., 2008). Interestingly, hERG1 is highly expressed in BOe, i.e. the most frequent precancerous lesion for OeA, while absent in healthy oesophageal mucosa and in oesophagitis (Lastraioli et al., 2006) (Fig. 1).

hERG1 expression in BOe is significantly associated to a higher risk to progress towards adenocarcinoma (Lastraioli et al., 2016). In the stomach, the hERG1 protein is not detected in the healthy lining epithelium of the normal mucosa while present in parietal (oxyntic) cells (Crociani et al., 2014a). hERG1 expression is switched on in precancerous lesions, in particular in gastric dysplasia (Fig. 1). Also in this case, hERG1 is associated to a higher risk of progression towards GC (Lastraioli, Romoli et al., 2019). Overall, hERG1 expression is apparently switched on in early phases of gastric carcinogenesis, as confirmed by a multicentric study on a wide Italian cohort of surgically resected patients with GC, which showed that hERG1 expression positively correlates with low grading and early (TNM I and II) stages of the Lauren's intestinal type of GC (Crociani et al., 2014b). Moreover, in the early stage, T1 patients, hERG1 expression identified high risk patients (Crociani et al., 2014a). Consistent with this, hERG1 mainly regulates cell proliferation through the regulation of vascular endothelial growth factor (VEGF) secretion (Crociani et al., 2014a). In the colon–rectum, hERG1 is not expressed in healthy colonic mucosa and sigma diverticulitis (Dolderer et al., 2010; Lastraioli et al., 2004). Precancerous colon rectal adenoma lesions express the channel, although expression levels increase in high-grade dysplastic colon rectal adenomas (Lastraioli et al., 2022). On the other hand, both the *KCNH2* transcript and the hERG1 protein are highly expressed in CRC cell lines and primary samples (Lastraioli et al., 2004). In CRC cells hERG1 is associated with sensitivity to chemotherapeutic drugs, including macrolide antibiotics, for example clarithromycin (Petroni et al., 2020), which are known to block hERG1 currents (Vandenberg et al., 2012), as well as with increased migration and invasiveness (Lastraioli et al., 2004). Consistent with this, hERG1 blockade *in vivo* (in mice) reduces tumour growth, invasiveness and metastatic spread (Crociani et al., 2013). These effects can be traced back to the formation of a complex between hERG1 channels and the β1 subunit of integrin receptors (Crociani et al., 2013) regulating hypoxia-inducible factor (HIF)-1α activation and VEGF-A secretion, whose characteristics are detailed below. hERG1 has turned out to be highly expressed in human primary CRC samples (Lastraioli et al., 2004), where the channel represents an independent negative prognostic factor in TNM I and II CRC when associated with Glut-1 absence (Lastraioli et al., 2012;

Muratori et al., 2016), and a prognostic biomarker to select metastatic CRC patients suitable to be treated with bevacizumab, when expressed in conjunction with HIF-2α (Iorio, Lastraioli et al., 2020). In normal pancreas hERG1 is absent in epithelial duct and acinar cells, while expressed at good levels in insulin secreting β-cells, where the channel is functional and negatively regulates cell firing and insulin secretion (Rosati et al., 2000). hERG1 expression is highly upregulated in human PCs, both the neuroendocrine (pNET) (Iorio et al., 2022)

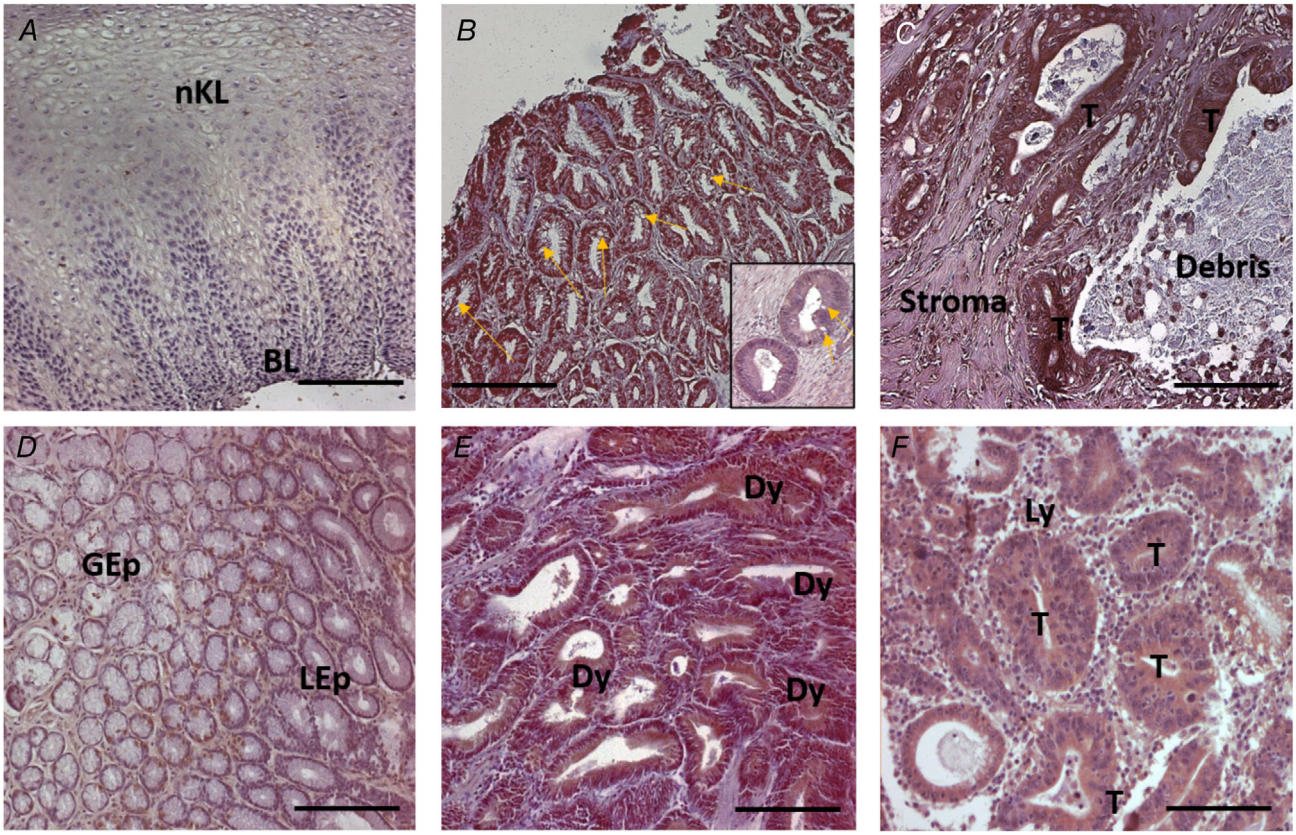

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Figure 1. hERG1 expression in representative images of human oesophageal and gastric samples
A, healthy oesophageal epithelium. hERG1 channel is absent in both the basal layer (BL) and the non-keratinised layer (nKL). *B*, Barrett's oesophagus with intestinal metaplasia highlighted by goblet cells (yellow arrows). hERG1 expression is present throughout the metaplastic glands, as witnessed by the brown precipitate due to diaminobenzidine peroxidation. The inset shows a higher magnification image of a representative sample in which goblet cells are indicated by yellow arrows. *C*, oesophageal adenocarcinoma. hERG1 expression is high in the tumour tissue (T), although a faint staining can be observed also in the stromal compartment and in the debris that might be present. *D*, healthy gastric epithelium. hERG1 channel is absent in both the lining epithelium (LEp) and the glands (GEp). *E*, gastric dysplasia. hERG1 is highly expressed in the dysplastic glands (Dy). *F*, gastric adenocarcinoma. hERG1 is highly expressed in the tumour tissue (T), and a faint staining can be observed also in the stroma due to the presence of lymphocytes (Ly) and other white blood cells. Scale bar: 100 µm (magnification ×20 (*A–F*) or ×40 (inset to *B*)). Immunohistochemistry was performed on 7-µm sections dewaxed and rehydrated, using an anti-hERG1 monoclonal antibody directed against the S5-pore region (MCK Therapeutics, Florence, Italy; patent number IT1367861) at 1:200 dilution (overnight at 4°C), after performing antigen retrieval with proteinase K incubated at 37°C for 5 min. Immunostaining was performed with a commercially available kit (PicTure Max kit and 3,3′-diaminobenzidine, Thermo Fisher Scientific, Waltham, MA, USA). All the representative slides shown in this figure were processed in Prof. Arcangeli's laboratory by one of the co-authors of the present review (E.L.). The study was conducted in accordance with the *Declaration of Helsinki*, and approved by the local Ethical Committee (BIO.14.033; 33.16TS).

and PDAC (Feng et al., 2014; Lastraioli, Perrone et al., 2015). In PDAC, hERG1 positivity occurs at high levels in roughly 60% of the samples and correlates with a high proliferation index, tumour grading and epidermal growth factor receptor (EGF-R) expression (Lastraioli, Perrone et al., 2015). Furthermore, hERG1 positivity represents an independent negative prognostic factor, in addition to the TNM tumour stage (Lastraioli, Perrone et al., 2015). In contrast, in pNETs hERG1 is mainly overexpressed in low grade and stage tumours, and represents a positive prognostic factor (Iorio et al., 2022). While hERG1 was not studied in human pancreatic pre-neoplastic lesions, the analysis conducted in KPC transgenic mice, a widely used model of PDAC, showed that the channel is expressed in PanIN, with expression scores increasing along with the progression from less to more severe lesions (Lastraioli, Perrone et al., 2015).

In summary, hERG1 is expressed both in precancerous lesions and in advanced tumours of the GI tract. In precancerous lesions, for example, BOe and gastric dysplasia, hERG1 overexpression represents a progression biomarker, contributing to identifying high risk patients, who must hence be submitted to a more stringent surveillance. In CRC and PDAC, hERG1 expression progressively increases during tumour progression (which is mirrored by the TNM stage) and behaves as a negative prognostic factor. Hence, hERG1 positive patients must be considered 'high risk' patients, even when belonging to low TNM stage, and treated accordingly. The exception of pNETs is intriguing: these tumours derive from the neoplastic transformation of neuroendocrine cells which express hERG1 physiologically. Therefore, in pNETs, hERG1 mainly represents a differentiation marker, and its expression has a positive impact on survival.

The relevance of the hERG1–β1 integrin complex in GI tumours

As described above, hERG1 controls different aspects of tumour behaviour, for example, cell proliferation and survival, cell migration and invasion, in all the tumours of the GI tract, with some specificities. For example, both in GC and CRC hERG1 modulates tumour progression by switching the VEGF-A-dependent angiogenetic pathways on (Crociani et al., 2013). Consistent with this, blocking hERG1 *in vivo* (in mice bearing GC cell xenografts) impairs tumour growth, angiogenesis and metastasis formation (Crociani et al., 2014a). In PDAC cells, hERG1 interacts with the EGF-R, which in turn affects EGF-R-dependent phosphorylation of extracellular signal-regulated kinase (ERK) 1 and 2, key signalling proteins downstream to EGF-R involved in cell proliferation (Lastraioli, Perrone et al., 2015). In PDAC cells hERG1 also regulates cell migratory programmes by modulating F-actin dynamics and organisation (Manoli et al., 2019). A unifying mechanism in the hERG1 role in neoplastic progression of GI tumours may indeed be constituted by the functional and physical link which occurs between the channel and adhesion receptors of the integrin family. After the first demonstrations in neuroblastoma and leukaemia indicating that integrin activation leads to increased hERG1 currents (Arcangeli et al., 1993, 1995), it emerged that the channel and the β1 subunit of integrins could also assemble onto the plasma membrane to give rise to a functional multiprotein complex (Cherubini et al., 2002, 2005). The hERG1–β1 integrin complex does not occur in the heart, where hERG1 assembles with its canonical accessory subunits, for example, the KCNE1 protein (Becchetti et al., 2017). Afterwards it became clear that the hERG1–β1 integrin complex is present exclusively in tumours, where it represents a novel tumour antigen, and is present in those advanced cancers where hERG1 overexpression was detected. This is evident Fig. 2, where one CRC (Fig. 2*A* and *B*) and one PC (Fig. 2*C* and *D*) sample that express hERG1 and hERG1–β1 integrin complex in the same cancer cells.

Depending on the pathophysiological context, the interplay between hERG1 and the integrin subunit can modulate different cancer-related signalling pathways, such as the phosphorylation of focal adhesion kinase (FAK) and ERK, the activation of phosphoinositide 3-kinase (PI3K) and the subsequent phosphorylation of AKT, the nuclear translocation of nuclear factor κB (NF-κB) and HIF-α, or the activation of small GTPases and the modulation of F-actin organisation and dynamics (Becchetti et al., 2019, 2022). When the interplay between the integrin and the channel involves the activation of K^+ (and current) flow through the open channel, mainly FAK and ERK phosphorylation are switched on, and cell proliferation mechanisms are triggered. This apparently occurs mainly in those tumours/precancerous lesions where hERG1 mainly regulates cell proliferation (Becchetti et al., 2017, 2019). In some circumstances, downstream signalling steps appear to require the formation of multiprotein complexes, which is a suggestion of signal transfer by conformational coupling (Becchetti et al., 2022). This has been proven in the case of the AKT-centred pathways. In CRC cells, for example, after cell adhesion to the extracellular matrix and integrin activation, hERG1 physically associates with the p85 subunit of PI3K, which stimulates AKT phosphorylation. The supramolecular complex thus regulates cell migratory and invasive programmes as well as autophagy (Becchetti et al., 2022). Interestingly, hERG1 associates with β1 integrin prevalently in the closed state (Becchetti et al., 2022). This points to a non-conductive function of hERG1, which transduces by conformational coupling the integrin-dependent

microenvironment signals, without being a canonical mechanosensitive channel (in that channel opening is not stimulated by membrane strain). Extremely relevant from the therapeutic point of view is the fact that the hERG1–β1 integrin complex can be specifically harnessed in its functions by a newly developed bispecific antibody, directed against the β1 integrin and the hERG1 protein, which selectively targets the two proteins once complexed. Such an antibody, in the form of a single chain Diabody (Duranti, Lastraioli et al., 2021) shows a good toxicological, biodistributional and therapeutic profile, especially in CRC and PDAC (Duranti, Iorio et al., 2021). In particular, no cardiac toxicity of the single-chain diabody emerged either in mice (Duranti, Iorio et al., 2021) or in humans (Arcangeli, 2021).

Further recent evidence better clarifies the oncological relevance of the hERG1–β1 integrin complex, which can recruit other ion channels and transporters in cancer cells and appears to be mostly implicated in controlling cell motility and migration. In PDAC cells, the channel sustains pro-metastatic signals through a reorganisation of F-actin in filopodia (Manoli et al., 2019). This effect can be traced back to the association of the hERG1–β1 integrin complex with small GTPases, mainly RhoA (Cherubini et al., 2005). This mechanisms can also involve the modulation of $[Ca^{2+}]_i$ (Fig. 3).

The hERG1–β1 integrin complex can also recruit the chemokine receptor CXCR4, to trigger AKT phosphorylation and cell migration (Fig. 3) (Pillozzi et al., 2011). In CRC cells, the β1 integrin-mediated adhesion

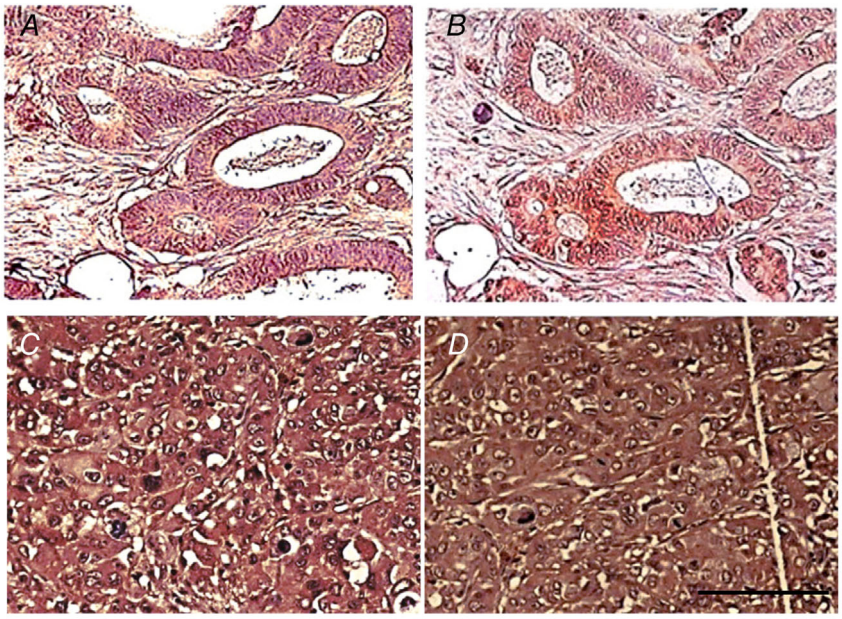

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Figure 2. Representative images of CRC and PDAC samples expressing hERG1 and hERG1–β1 integrin complex
A, hERG1 positive CRC sample. *B*, hERG1–β1 integrin complex positive CRC sample. *C*, hERG1 positive PDAC sample. *D*, hERG1–β1 integrin complex positive PDAC sample. Scale bar: 100 µm (magnification ×20). Immunohistochemistry was carried out on 7-µm sections on positively charged slides. After dewaxing and dehydrating the sections, endogenous peroxidases were blocked with a 1% H_2O_2 solution in phosphate-buffered saline. Subsequently, antigen retrieval was performed by treatment with proteinase K at 37°C for 5 min. The following antibodies were used at the dilutions reported in parentheses: anti-hERG1 monoclonal antibody (0.005 µg/µl; MCK Therapeutics, patent number IT1367861) and scDb hERG1–β1 (20 µg/ml; MCK Therapeutics, patent number IT102017000083637). Incubation with the primary antibody was carried out overnight at 4°C. For the scDb hERG1–β1, anti-his antibody was incubated for 90 min at room temperature. Immunostaining was performed with a commercially available kit (PicTure max kit; Thermo Fisher Scientific) according to the manufacturer's instructions. All the representative slides shown in this figure were processed in Prof. Arcangeli's laboratory by one of the co-authors of the present review (J.I.). The study was approved by the local Ethical Committee (BIO.14.033; 33.16TS). All the patients were enrolled after informed written consent and the study was performed in accordance with the *Declaration of Helsinki*.

increases the values of intracellular pH by activating the Na^+/H^+ transporter NHE1, which assembles with the $\beta1$ integrin and hERG1. The tri-molecular complex sustains CRC cell motility (Iorio, Duranti et al., 2020) (Fig. 3). The hERG1–$\beta1$ integrin complex can recruit another transporter, carbonic anhydrase IX (CAIX; Lastraioli, Pillozzi et al., 2019). This tri-molecular complex has been shown to occur in clear-cell renal carcinoma (ccRC) cells (Fig. 3), but preliminary experiments suggest its occurrence also in CRC and GC cells, where a strong association between hERG1 and CAIX occurs in primary samples (Iorio, Lastraioli et al., 2020; Lastraioli et al., 2012; Muratori et al., 2016). The hERG1–$\beta1$ integrin–NHE1 and hERG1–$\beta1$ integrin–CAIX complexes presumably constitute functional hubs to drive extracellular matrix-triggered variations in intra- (and extra-) cellular pH, especially in hypoxic conditions, which drive pro-metastatic signalling pathways. In this scenario, hERG1 would represent the link between the metabolic switch which occurs in cancer cells and enables them to thrive in an acidic and hypoxic tumour microenvironment, through both the presence of a PAS domain (i.e. a protein domain capable of directly sensing P_{O_2}) in its N-terminus (Vandenberg et al., 2012), and the activity of the hERG1–$\beta1$ integrin complex

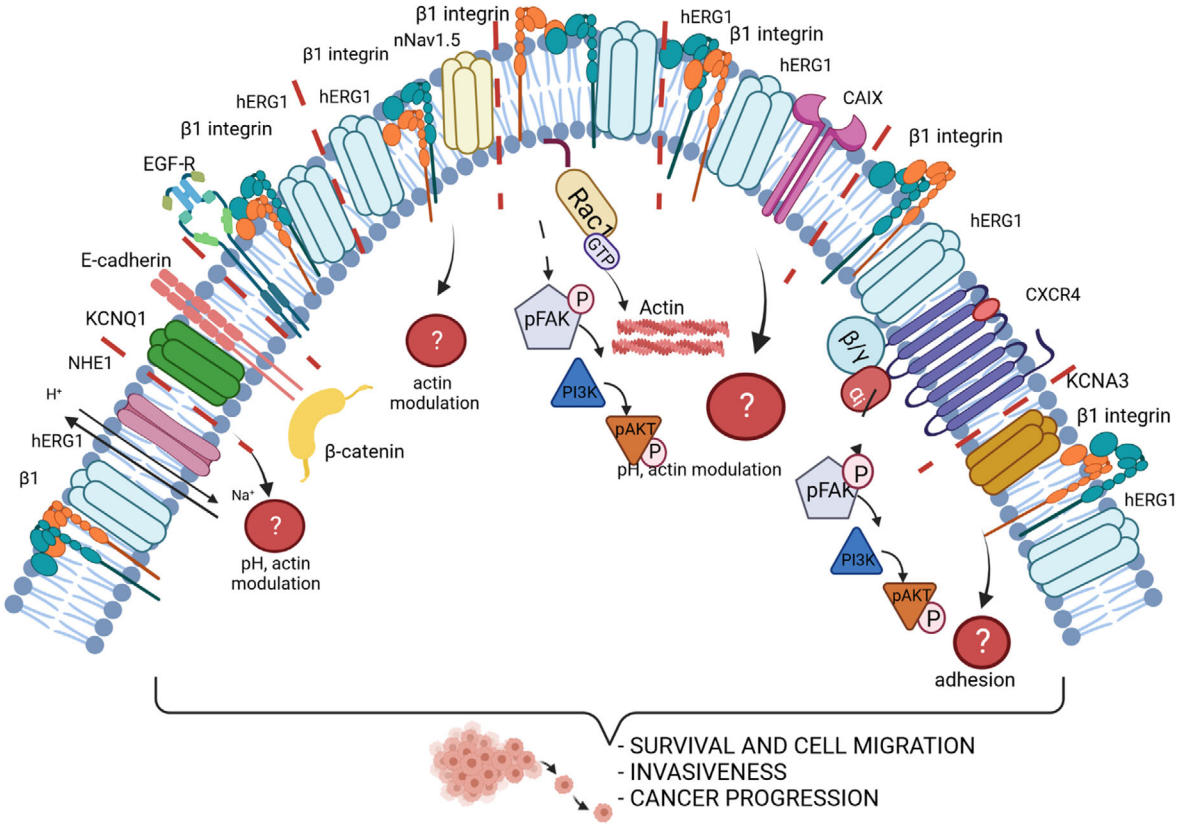

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Figure 3. Macromolecular complex-mediated signalling in relation to hERG1 and other voltage gated ion channels
Different ion channel complexes mediate signalling pathways and control different aspect of cancer cell behaviour, such as proliferation, survival, invasiveness and progression. The figure shows different macromolecular complexes mediated by hERG1 and its interaction with $\beta1$ integrin differentiated by red dotted lines, from left to right: hERG1–$\beta1$ complex with NHE1 exchanger responsible for pH and actin modulation; hERG1–$\beta1$ complex with EGF-R; KCNQ1 and E-cadherin, which modulates β-catenin; hERG1–$\beta1$ complex with nNav1.5 sodium channel, which is involved in cell proliferation; hERG1–$\beta1$ complex *per se*, which modulates downstream signalling involving FAK, AKT and Rac1; hERG1–$\beta1$ complex with carbonic anhydrase IX (CAIX); hERG1–$\beta1$ complex with C-X-C chemokine receptor type 4 (CXCR4); and hERG1–$\beta1$ complex with KCNA3. Created with BioRender.com.

on HIF(s) expression through the PI3K–AKT–NF-κB pathway (Crociani et al., 2013; Iorio et al., 2019). Finally, the hERG1–β1 integrin complex can also include the neonatal form of the voltage-dependent sodium channels (nNa_V 1.5) (J. Iorio, unpublished data), which are over-expressed in breast cancer (Fraser et al., 2005) and CRC (Lastraioli et al., 2021).

The ion channel complex landscape

Ion channels operate, in excitable and non-excitable cells, in a highly coordinated manner, within well organised functional networks, which may also involve the formation of multi-channel complexes. Such complexes also occur and are operative in cancer cells, with some interesting differences, which make ion channel complexes highly relevant in tumour pathophysiology. There are clear examples of multi-channel functional complexes with regard to calcium signals. Store-operated calcium entry in response to calcium depletion in intracellular stores is typically mediated by the plasma membrane Orai1 channels, which are activated by STIM1 proteins, at the endoplasmic reticulum–plasma membrane contact sites, through conformational coupling (Yu & Machaca, 2022). In excitable cells, the big conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) and Ca_V channels associate, which can allow localised Ca^{2+}-dependent regulation of K^+ channels during action potential repolarisation (Sancho & Kyle, 2021). A similar mechanism is also operative for K_V1.3 channels, which form complexes with different Ca^{2+} channels, with the effect of amplifying Ca^{2+} flux. In cancer cells, both voltage-dependent and -independent Ca^{2+} channels interact with K^+ channels, giving rise to complexes which are regulated by several molecules including proteins (e.g. STIM), receptors (e.g. S1R/SIGMAR1) and lipids (mainly ether lipids and cholesterol) (Potier-Cartereau et al., 2022).

In the heart, the inwardly rectifying potassium channel K_{IR}2.1 and Na_V1.5 interact within a macromolecular complex, modulating their respective expression levels, thereby contributing to modulation of cardiac excitability (Ponce-Balbuena et al., 2018). Similarly, the transcripts encoding Na_V1.5 and hERG1 (*SCN5A* and *KCNH2*, respectively) have been reported to be associated in 'microtranslatomes', that is, complexes occurring during protein translation. The *SCNA5*/*KCNH2* 'microtranslatome' serves to coordinate the expression levels of the two currents in the heart (Eichel et al., 2019). A similar situation apparently occurs in breast cancer cells, where the two transcripts (and related proteins) are upregulated in parallel (J. Iorio, unpublished data), but in this case the 'neonatal' form of SCNA5 is involved. hERG1 is involved also in another interesting balancing of channel-encoding transcripts: in CRC cells hERG1 blockade leads to increased K_{Ca}3.1 expression, both transcript and protein, which in turn increases cisplatin uptake and in turn induces apoptosis (Pillozzi et al., 2018).

Other functional complexes involve ion channels and transporters to give rise to the so called 'chansporter' complexes. Examples are the complexes between different K_Vs and sodium-coupled solute transporters in neurons (Manville & Abbott, 2019) and heteromeric KCNQ2–KCNQ3 (Kv7.2/7.3) (i.e. the neuronal M-current) with two sodium-coupled neurotransmitter transporters, DAT and GLT1 (Manville & Abbott, 2019). Cumulative evidence suggests that 'chansporter' complexes represent a widespread form of cellular signalling hub, in the CNS and other tissues. In GI tumours 'chansporters' between hERG1 and NHE1, or hERG1 and CAIX, which also include the β1 integrin, regulate intracellular pH and cell migration (Iorio, Durant et al., 2020).

The multiple channel–transporter complexes are added to those between ion channels and their canonical accessory subunits, which are necessary to fine tune channel activity. Na_V channels, for example, are complexed with accessory β subunits, which not only modulate channel gating and kinetics, but also represent multifunctional signalling molecules involved in cell adhesion, cell migration and neurite outgrowth (Bouza & Isom, 2018). Interestingly, mutations in the β subunit genes (*SCN1B–SCN4B*) have been linked to a variety of diseases, including cancer (Roger et al., 2015). KCNQ1 channels associate with KCNE1 to generate the slow delayed rectifier (IKs) current in the heart (Hasani et al., 2018), as well as with either KCNE2 (gastric) or KCNE3 (intestinal), which apparently convert from a voltage-dependent channel in the stomach into a voltage-independent, constitutively open channel in the intestine (Anderson et al., 2019). This has a clear functional impact, since the KCNQ1–KCNE2 complex is essential for gastric acid secretion, whereas the basolateral KCNQ1–KCNE3 establishes the driving force for cAMP-mediated Cl^- secretion through cystic fibrosis transmembrane conductance regulator, necessary for mucus hydration, in intestinal crypts (Anderson et al., 2019). This scenario changes in tumours of the GI tract, where KCNQ1 forms a complex with β-catenin, which is hence sequestered at the plasma membrane level. This limits β-catenin transcriptional activity. This mechanism is relevant in CRC, where *KCNQ1* behaves as a tumour suppressor, presumably thanks to this change in the function of β-catenin (Anderson et al., 2019). Similarly, KCNE1 forms complexes with hERG1 channels in the heart (Vandenberg et al., 2012) while it is replaced by the β1 subunit of integrin receptors in tumours. This substitution is responsible for improving the functioning of hERG1 as a non-conductive signalling molecular device

(Becchetti et al., 2017). Quite similar is the situation of Kv1.3, which associates with the KCNE4 accessory subunit to trigger leukocyte activation (Immler et al., 2022) and complexes with the β1 subunits of integrins to regulate migration in cancer cells (Artym & Petty, 2002).

Conclusion: the channel complexes landscape in tumours

During neoplastic transformation, ion, in particular potassium, channels are upregulated and often operate within macromolecular complexes, as occurs for ion channels expressed in normal excitable and non-excitable tissues. However, channel macromolecular complexes in tumours often comprise 'non canonical' proteins. A prototype is represented by hERG1 and its propensity to form complexes with cell adhesion receptors (e.g. integrins) or growth factor receptors (e.g. EGF-R), besides different ion transporters or channels. Another peculiar aspect of channel complexes in tumours is their localisation in those highly dynamic membrane microdomains called lipid rafts. Lipid rafts are known to determine several cellular functions including cell migration and invasion (Greenlee et al., 2021). Recently, the hERG1–β1 integrin complex has been shown to specifically localise in lipid rafts in PDAC cells (C. Duranti et al., unpublished results). Finally, when ion channels operate within multiprotein complexes in tumours, they often work in a non-conductive way, to trigger and activate intracellular signalling cascades mainly through, for example, conformational coupling (Forzisi & Sesti, 2022). We have provided evidence for hERG1, which operates in tumours either as a conductive channel (to trigger FAK and ERK phosphorylation and activation) or within integrin complexes as a closed channel to activate the PI3K–AKT signalling pathway (Becchetti et al., 2017). This is reminiscent of what was recently shown for SWELL1 (LRRC8A), which can switch its canonical function as a swell-activated anion channel to a signalling hub that regulates the PI3K–AKT signalling pathway in either skeletal muscle or endothelial cells in pathological conditions, such as type 2 diabetes (Kang et al., 2018).

From a pharmacological standpoint, the multiprotein complexes described above offer unique opportunities for cancer cell targeting. The latter can be achieved by using, for example, bispecific antibodies which can simultaneously bind two or more proteins (Duranti & Arcangeli, 2019; Duranti, Iorio et al., 2021), impairing downstream signalling pathways. In addition, the occurrence of non-conductive channels in multiprotein complexes in tumours paves the way for targeting different channel conformational states. Overall, we favour the possible exploitation of the hERG1–β1 complex as a molecular target for therapeutic purposes with a specific therapeutic antibody (the single-chain diabody hERG1–β1) which impacts on cancer-relevant signalling pathways, such as AKT and HIF-1α, which control proliferation, migration and cancer progression (Duranti, Iorio et al., 2021). In this regard, a proper validation of this new tool is mandatory to better outline the possible therapeutic application, alone or in combination with standard drugs such a gemcitabine (Lottini et al., 2022) or bevacizumab (Lottini et al., 2020).

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Additional information

Competing interests

None.

Author contributions

A.A. designed, wrote and supervised the manuscript; C.D. wrote the manuscript and prepared the figures; J.I. prepared the figures and wrote the manuscript; E.L. performed bibliographical researches, prepared the figures and wrote the manuscript. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Supporting information

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