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hERG1 Channels in Human Esophagus: Evidence for Their Aberrant Expression in the Malignant Progression of Barrett's Esophagus

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Ion channels regulate a broad range of cellular activities. Alteration in ion channel function has been reported in different human pathologies, such as cardiac, neuromuscular, autoimmune diseases, and cancer. We investigated the expression of hERG1 K^+ channels in the human upper gastrointestinal tract, focusing our attention on the lower esophagus. In particular, we analyzed by both Reverse transcription and polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) endoscopic samples obtained from normal subjects, from patients suffering from gastroesophageal reflux, associated or not with esophagitis, and from patients affected by Barrett's esophagus (BE), that is, intestinal metaplasia. None of the normal samples, nor those from patients with gastro-esophageal reflux symptoms and reflux esophagitis expressed the hERG1 protein. On the other hand, 69% of patients with BE expressed hERG1. Since BE is a preneoplastic lesion, dysplasias (Ds) and adenocarcinomas (ADKs) arising on a previously diagnosed BE were also analyzed, and all the samples showed a high expression of the hERG1 protein. The surveillance of patients with BE showed that 89% of those who later developed ADKs displayed hERG1 expression. Data here reported, support the hypothesis that hERG1 expression marks an early step of the progression of normality to cancer in the human esophagus through a metaplastic and dysplastic stage. J. Cell. Physiol. 209: 398–404, 2006. © 2006 Wiley-Liss, Inc.

Increasing evidence has recently addressed an altered expression of ion channel encoding genes, as well as ion channels malfunction as an important step in the development and progression of several human diseases, from cardiac, neuromuscular, and autoimmune diseases to cancers (Ashcroft, 2000). The role of ion channels in pathophysiological processes is as diverse as the ion channels themselves, ranging from the regulation of cellular excitability and secretion to the control of cell proliferation and survival (Arcangeli and Becchetti, 2005). Potassium channels encoded by the *human ether-a-go-go related gene 1* (*herg1*) (hERG1 channels) are involved in several human diseases, from arrhythmias (Sanguinetti and Tristani-Firouzi, 2006), to hyperthension (Sarzani et al., 2006). Moreover, we have repeatedly reported that the mis- and over-expression of hERG1 channels occurs in many types of human cancers (Arcangeli, 2005). No indications have been gathered so far regarding the expression of hERG1 channels in inflammatory and preneoplastic conditions.

We, therefore, designed a study to investigate the expression of hERG1 channels in pathologic conditions of the human upper gastrointestinal tract, with particular attention to esophageal diseases like the gastro-esophageal reflux disease (GERD) and the so called Barrett's esophagus (BE). BE is an acquired condition that results from GERD and is characterized by the metaplastic replacement of the normal squamous epithelium of the lower esophagus, up to the squamocolumnar junction (also known as Z line), by a columnar, intestinal-like, epithelium (Spechler, 2002). In other words, when defence mechanisms in esophageal mucosa are chronically overwhelmed by harmful agents, BE develops as a healing process protecting the esophagus from further damage (Guillem, 2005). BE is a premalignant condition that predisposes to the development of esophageal adenocarcinoma (Haggitt and Dean, 1985), atumor with an increasing frequency in most western countries (Newnham et al., 2003). Esophageal adenocarcinomas (ADKs) may develop from BE through a multistep morphological pathway (Fléjou, 2005). This

Abbreviations: ADK, adenocarcinoma; BE, Barrett's esophagus; D, dysplasia; E, esophagitis; GERD, gastro-esophageal reflux disease; IHC, immunohistochemistry; N, normal esophagus; PCR, polymerase chain reaction; RT, reverse transcription.Paolo Bechi and Annarosa Arcangeli contributed equally to this work.

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process is characterized by increasing grades of dysplasia (D) (intraepithelial neoplasia) that precede the onset of an invasive adenocarcinoma (Montgomery et al., 2001) and are paralleled by the accumulation of genetic abnormalities (Jankowski et al., 1999; Merola et al., 2006). Among these genetic changes, mutation of the human suppressor gene *p53* have been reported to characterize BE, and have been, therefore, proposed as an adjunct to morphology for the screening and surveillance of patients with BE (Merola et al., 2006).

We studied the expression of the *herg1* gene and of the hERG1 protein in endoscopic samples obtained from patients suffering from BE. Such expression was compared with that occurring in samples of normal esophageal mucosa, as well as of mucosa from patients with symptoms of GERD with or without histologically confirmed esophagitis (E). All the endoscopic samples were taken from the lower esophagus, below the squamocolumnar junction; that is, the areas where the intestinal metaplasia characterizing BE arises. Finally, the expression of the hERG1 protein was evaluated on samples of Ds and ADKs arising from a previously diagnosed BE.

MATERIALS AND METHODS

Patients

Ninety patients (45 males and 45 females; mean age 55.8, range 29–86) were enrolled in the study. All the patients underwent endoscopic examinations due to gastroesophageal reflux disease (GERD) symptoms and/or previous BE diagnosis, based on endoscopic and histological features. Patients were treated at the Patologia Chirurgica 1 of the University of Florence and the I Divisione Clinicizzata di Chirurgia Generale of the University of Verona. In each patient, three adjacent samples were taken immediately below the squamocolumnar junction during the endoscopic examination by means of grasp forceps. One was immediately frozen for RNA extraction and the other two were fixed in 4% formaldehyde in PBS to be embedded in paraffin for immunohistochemistry (IHC) and for conventional histology, respectively. In all the patients, an additional biopsy above the squamocolumnar junction was taken to assess the presence or the absence of the pathologic features of reflux E (hyperplasia of squamous epithelium, papillary vascular ectasia, presence of inflammatory cells). Histological diagnoses were assessed by LM (Florence) and AT (Verona), respectively through conventional techniques such as Hematoxylin-Eosin staining and Alcian Blue Ph 2.5-PAS. Two groups were distinguished on the basis of the histopathologic findings in the biopsy specimen which was collected above the squamocolumnar junction: no histologic lesions (31 specimens from patients with GERD symptoms but no histologic E, addressed as GERD) and E (40 specimens from patients with both symptoms and histologic reflux E, addressed as GERD plus E). This type of biopsy was also collected in the patients belonging to the BE group (13 specimens), and analyzed as above; however, such biopsy had no relevance to the definition of the group, which was identified on the basis of the currently accepted criterion of the presence of intestinal metaplasia in the biopsy collected below the squamocolumnar junction and above the gastroesophageal junction. Finally, endoscopic samples were collected from six patients without reflux symptoms undergoing upper endoscopy for different reasons (two gastric leiomiomas, three peptic ulcers, one gastritis). Also in this case, biopsies were collected both above and below the squamocolumnar junction, and the above methodological criteria were applied. These specimens did not show any histologic lesion in the esophageal biopsies either above or below the squamocolumnar junction and were, therefore, considered as controls for the purpose of the study, and addressed as "normal esophagus" (N).

A retrospective immunohistochemical analysis was performed on 6 BE-associated Ds and 8 BE-derived esophageal ADKs, as well as on 27 cases of BE for which a 5-year follow-up was available, and were, therefore, differentiated as non progressing, or progressing to a pure adenocarcinoma. Finally, archival specimens of two patients where the Barrett's esophagus-dysplasia-adenocarcinoma sequence was followed, were analyzed.

Reverse transcription (RT) and polymerase CHAIN reaction (PCR)

Total RNA was extracted as previously reported (Cherubini et al., 2000). Briefly, all the samples were homogenized in a Guanidine Thyocianate solution and total RNA was extracted using Phenol and Chloroform-Isoamylic alcohol standard protocol. RNA (2 μg) was then retrotranscribed as previously described (Pillozzi et al., 2002) using 200 U of Superscript II Reverse Trascriptase (Invitrogen, Milan, Italy) and random hexamers (2.5 mM) in a 20 μl reaction, according to the manufacturer's protocol.

cDNA (1 μl) was then amplified by PCR in a 50 μl reaction using Platinum PCR Supermix (Invitrogen) and the following primers (Cherubini et al., 2000) at a concentration of 100 ng/μl:

herg1 forward: 5′-TCCAGCGGCTGTACTCGGGC-3′.
herg1 reverse: 5′-TGGACCAGAAGTGGTCGGAGAACTC-3′.
gapdh forward: 5′-AACAGCCTCAAGATCATCAGCAA-3′.
gapdh reverse: 5′-CAGTCTGGGTGGCAGTGAT-3′.

The conditions for the amplification of *herg1* and *gapdh* genes were the same reported by Pillozzi et al. (2002): 2 min at 94°C, followed by 35 cycles composed of a denaturation step at 94°C for 30 sec, an annealing step at 56°C (*herg1*) or 60°C (*gapdh*) for 1 min, and an elongation step at 72°C for 1 min. An additional over-extension was then performed at 72°C for 2 min.

On representative samples Real Time PCR experiments using the TaqMan Gene Expression assay for human CD45 kit (Applied Biosystems, Foster City, CA) were performed, according to the manufacturer's instructions.

Immunohistochemistry (IHC)

IHC was performed as previously reported (Lastraioli et al., 2004) on 7-μm sections adhered on positive-charged microscope slides. After dewaxing and re-hydrating, the specimens were treated with a 1%H_2O_2 solution and antigen retrieval was carried out using a Proteinase K (Roche, Milan, Italy) solution (5 μg/ml in PBS). Permeabilization was performed with a 0.1% Triton X100 in UltraVBlock (LabVision, Fremont, CA) solution. The anti-hERG1 polyclonal antibody (Alexis Corporation, Lausen, Switzerland) was then applied and incubation was carried out at 4°C O/N. The antibody was diluted in UltraVBlock (LabVision): PBS 1:10 (v/v) at a 1:200. When dealing with bioptic or archival specimens, the antibody was diluted 1:200 and 1:500, respectively. In any case, preliminary experiments were carried out to test the best concentration of the anti-hERG1 antibody to be used, depending on the different fixatives used. Detection was carried out using a commercially available kit (PicTure Plus Kit, Zymed, Milan, Italy) as well as DAB chromogen solution (Zymed), according to the manufacturer's instructions. Every sample showing staining of more than 1% epithelial cells was considered as positive while samples negative for hERG1 staining in epithelial cells were considered as negative irrespective of immunoreactivity in non-epithelial (smooth muscle, inflammatory) cells.

An IHC using Mib-1 antibody specific for Ki-67 antigen (DakoCytomation, Milan, Italy) was performed on representative specimens. Antigen retrieval was carried out with microwave treatment in citrate buffer for 20 min; the primary antibody was used at a 1:50 dilution and incubated at 25°C for 1 h. Immunodetection was performed using EnVision kit (DakoCytomation) followed by DAB chromogen solution (DakoCytomation).

Evaluation of the results obtained through IHC experiments was carried out by two independent operators.

Statistical analysis

The relationships between *herg1* gene and hERG1 protein expression and clinico-pathological parameters were evaluated

through Fisher's exact test. P-values < 0.05 were considered statistically significant.

RESULTS

herg1 gene and hERG1 protein expression in normal and pathological human esophagus

herg1 gene expression was analyzed by PCR on 90 endoscopic samples collected at the Patologia Chirurgica 1 of the University of Florence and the I Divisione Clinicizzata of the University of Verona. Samples were divided into four groups: (i) N without GERD symptoms; (ii) GERD symptoms without histological esophagitis (GERD); (iii) GERD symptoms with esophagitis (GERD plus E); (iv) BE. All samples used for biomolecular and immunohistochemical (see below) analysis were collected below the squamocolumnar junction, that is, in the anatomical region where the BE disease arises, while the histopathological diagnosis of E and therefore, the attribution of the patients to the groups of GERD and GERD plus E was done on the biopsy sample collected above the squamocolumnar junction. The *gapdh* housekeeping gene was amplified as a control. Representative gels are shown in Figure 1: none of the samples belonging to N showed *herg1* expression (part A, lanes "N"). Similarly, no expression was detected in GERD samples (part A, lanes "GERD"). Examples of *herg1* transcripts amplified from samples of patients with GERD plus E are reported in part B; in this case, two out of four samples showed a *herg1*-specific band. Finally, examples of PCR amplification from BE samples are shown in part C, where *herg1* expression is evident in all the samples reported in the figure. Overall, PCR analysis showed that none of the normal samples and 6 out of 31 (20%) of GERD group expressed *herg1*. Among GERD plus E samples, the *herg1* transcript was detected in 13 out of 40 samples (32%), while the percentage of *herg1*-positive samples strongly increased (69%) in BE samples.

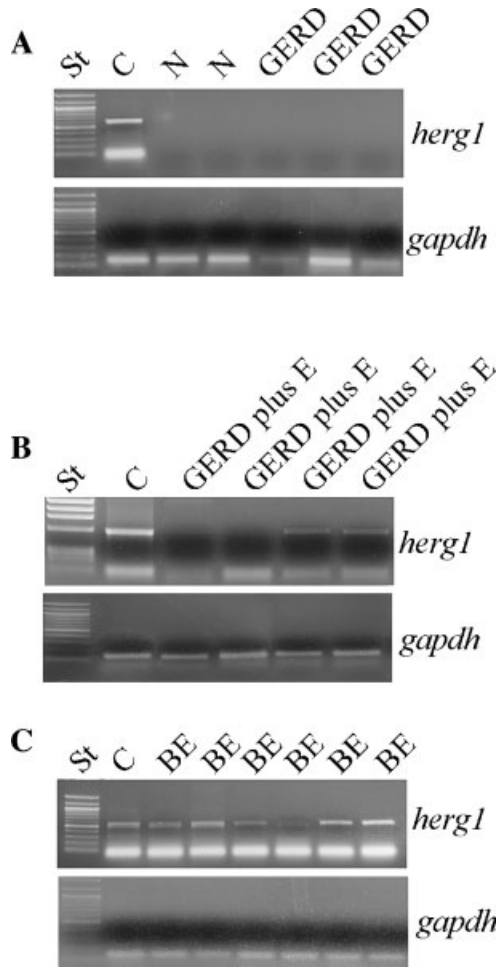

Fig. 1. RT-PCR on esophageal biopsies. Amplification on *herg1* (upper parts) and *gapdh* genes (lower parts) on: (**A**) normal esophagus samples (N) and specimens deriving from patient with GERD (**B**) biopsies of patients affected by esophagitis (GERD plus E); (**C**) Barrett's Esophagus samples (BE); FLG 29.1 cell line cDNA was used as a positive control (C); St: 100 bp, New England Biolabs.

Different cell types are present in the endoscopic samples used for the PCR analysis; among them smooth muscle cells, which highly express the *erg1* gene in the rat (Ohya et al., 2002), are represented. For this reason, the PCR analysis was paralleled by an IHC analysis to determine hERG1 protein expression at the histological level in the epithelial cells. Representative light micrographs of IHC analysis are reported in Figure 2 parts A–D, whereas the quantitative IHC results concerning all the samples are shown in Figure 3. No hERG1 expression was detected in normal samples either in the lower cell layers of the squamous epithelium (Fig. 2 part A) or in the cardiac-type epithelium present in samples collected below the squamocolumnar junction (Fig. 2B). Similar results were obtained in GERD samples. Samples from patients with GERD plus E were negative for hERG1 expression in epithelial cells, while an evident staining in inflammatory cells infiltrating the mucosa collected below the squamocolumnar junction was evident (part C). The specificity of the immunoreaction is more evident comparing this picture with that relative to the negative control (inset to part C). The same expression in inflammatory cells was also detected in samples collected above the squamocolumnar junction (and used for the diagnosis of E) (not shown). Conversely, samples from BE very often showed

Fig. 2. Immunohistochemistry for the hERG1 protein on esophageal biopsies. **A**: normal esophagus: squamous epithelium (taken above the squamocolumnar junction); note that the upper portion of the squamous epithelium shows an aspecific chromogen deposition in dead keratinized cells. The inset shows the negative control (i.e., IHC with no primary antibody in a different area of the same sample); (**B**) normal esophagus: columnar epithelium (taken below the squamocolumnar junction). The inset shows the negative control (i.e., IHC with no primary antibody in a different area of the same sample); (**C**) patient with esophagitis (note that the image here reported refer to the sample taken below the squamocolumnar junction, while the diagnosis of esophagitis was done on the sample taken above the squamocolumnar junction, see Materials and Methods). The inset shows the negative control (i.e., IHC with no primary antibody in a different area of the same sample); (**D**) Barrett's esophagus. The inset shows the negative control (i.e., IHC with no primary antibody in a different area of the same sample); (**E**) BE-derived dysplasia. The inset shows the negative control (i.e., IHC with no primary antibody in a different area of the same sample); (**F**) BE-derived adenocarcinoma. The inset shows the negative control (i.e., IHC with no primary antibody in a different area of the same sample); (**G**) Ki67 expression in (1) esophagitis; (2) Barrett's esophagus; (3) dysplasia; (4) Barrett's esophagus-derived esophageal adenocarcinoma. **H**: hERG1 expression through the metaplasia-dysplasia-adenocarcinoma sequence: (1) Barrett's esophagus; (2) dysplasia; (3) Barrett's esophagus-derived esophageal adenocarcinoma. IHC was performed on 7-μm sections. As described in Materials and Methods, the anti-hERG1 polyclonal antibody (Alexis Corporation) was routinely used at a 1:200 dilution. 1:500 dilution was used for archival specimens. As concerning IHC for Ki67, it was performed as described in Materials and Methods, with a 1:50 dilution. Bar: A 200 μm; B-H3 100 μm.

an intense hERG1 immunostaining (part D). In particular, immunolabeling of hERG1 protein demonstrated a strong chromogen deposition in metaplastic tissue; in most samples, hERG1 expression was diffused throughout the metaplastic glands, characterized by the presence of goblet cells (as shown in Fig. 2D). Alternatively, hERG1 positivity sometimes showed a focal pattern (see arrow in part G1). In any case, all the samples evaluated as "positive" showed an intense staining in the cytoplasm of metaplastic cells with respect to stromal cells (Fig. 2D). Here again, the specificity of the immunoreaction is more evident

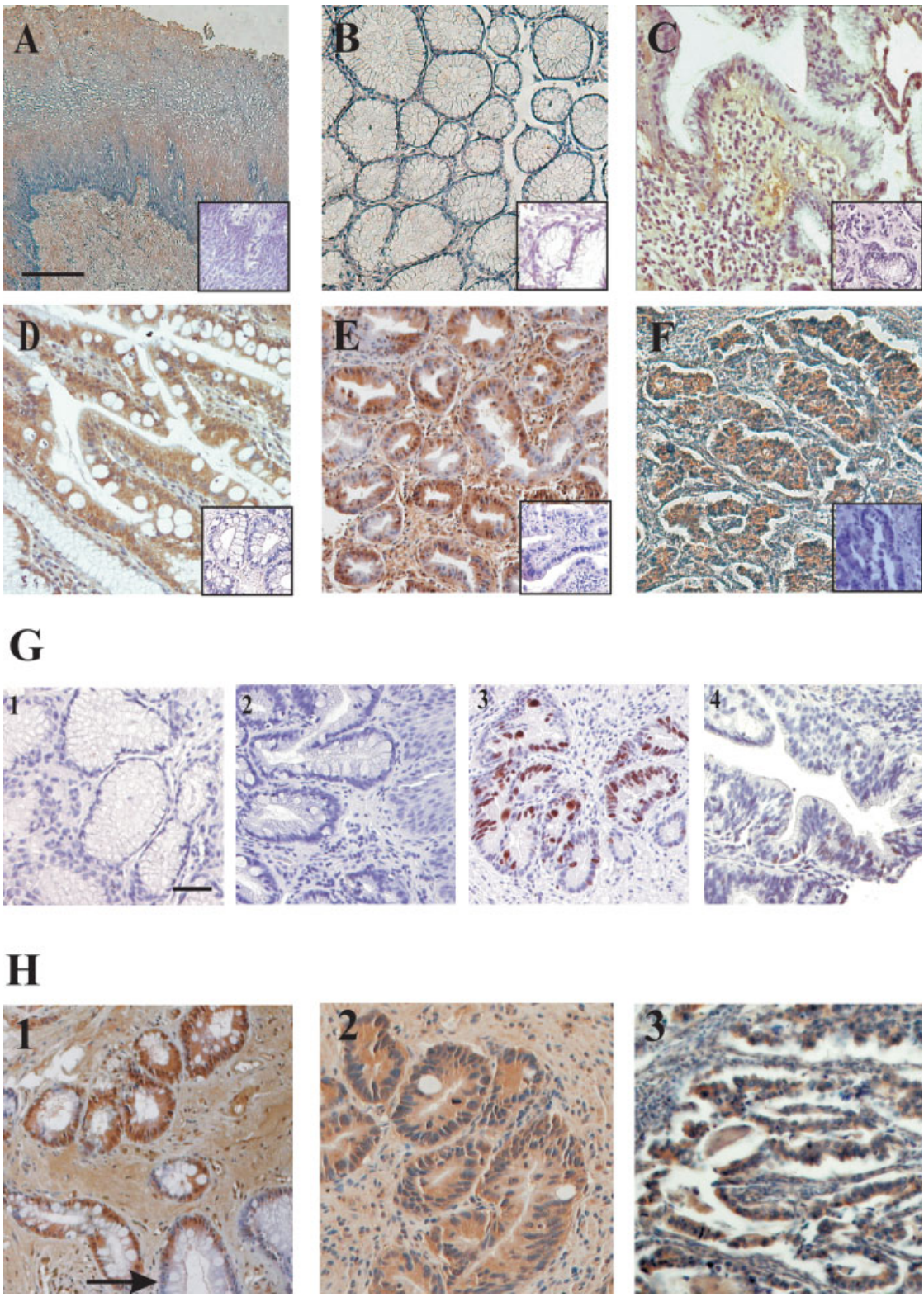

Fig. 2.

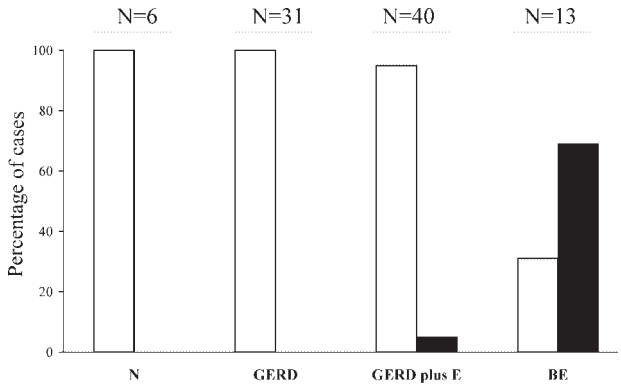

Fig. 3. hERG1 expression in various esophageal clinicopathological situations. Histogram relative to IHC results. Data are expressed as percentage of the total number of samples examined. N, normal esophagus (n = 6); GERD, symptoms of gastroesophageal reflux (n = 31); GERD plus E, symptoms of GERD with esophagitis (n = 43); BE, Barrett's esophagus (n = 21); D, Dysplasia (n = 6); ADK, Adenocarcinoma (n = 9). White bars, hERG1 negative samples; Black bars, hERG1 positive samples.

comparing this picture with that of the negative control (inset to part D). Results concerning IHC are summarized in Figure 3: no hERG1 expression was shown in the normal nor in the GERD group as well as in GERD plus E samples. On the other hand, 69% of BE samples were positive for hERG1 expression.

Overall, PCR and IHC results roughly overlapped in esophageal tissue as previously reported in other human normal and neoplastic tissues (Cherubini et al., 2000; Lastraioli et al., 2004). Discrepancies were observed in the groups of samples from patients with GERD and GERD plus E. In this group, a higher percentage of positive samples was detected by PCR when compared to IHC (see Table 1). This discrepancy would be due to the presence of either smooth muscle cells (see Discussion) or inflammatory cells infiltrating the tissue sample. This was confirmed by measuring *CD45* expression by quantitative Real Time PCR in representative GERD plus E samples that resulted to be either positive or negative for *herg1* expression at the PCR analysis. It emerged that *CD45* expression (normalized on the expression of the control gene, *gapdh*) was significantly higher in GERD plus E samples that were positive for *herg1* expression (2.21 ± 1.25 (n = 10) versus 0.215 ± 0.045 (n = 10);mean ± SE). This implies that such samples are more contaminated by inflammatory cells. On the whole, our results have shown that a high percentage of samples from patients affected by BE express the hERG1 protein, with significant differences between BE and all the other groups (Table 1).

hERG1 expression along the metaplasia-dysplasia-adenocarcinoma sequence

Since BE is thought to be the precursor lesion to esophageal ADKs, we sought whether hERG1 expression might represent one of the earliest steps leading esophageal epithelial cells to a cancerous state. Therefore, we performed a retrospective IHC analysis on six cases of Ds arising on a previously diagnosed Barrett's esophagus (BE-derived Ds) as well as on eight pure ADKs arising from a previous BE (BE-derived ADK). Representative examples of IHC performed on a dysplastic and an adenocarcinoma sample are reported in Figure 2, parts E and F, respectively. For comparison, pictures of the negative controls are reported in the insets. As previously reported for BE samples, hERG1 immunostaining was strong in the cytoplasm of neoplastic cells (Fig. 2F), while nuclei always turned out to be negative, staining blue. As concerning Ds, a diffuse pattern of expression was detected in the majority of samples, while a focal pattern was observed only in some of the samples (an example of which is reported in Fig. 2E). In any case, an intense hERG1 immunostaining of dysplastic cells with respect to the stroma was observed. In ADKs, the pattern of hERG1 expression was always of the diffuse type. Overall, the percentage of hERG1-positive samples raised to 100% in BE-derived Ds irrespective of the grade as well as in pure ADKs. Four samples displayed mixed histological features with both metaplastic and dysplastic areas; in these cases, the hERG1 protein was expressed in both types of lesions, with almost the same immunoreactivity. We also assessed whether hERG1 immunoreactivity in our samples overlapped with that of a proliferation-related antigen. To this purpose BE, dysplastic, and ADK samples were decorated with an anti-Ki67 antibody (Mib-1). Results reported in parts G1–G4 clearly showed that both GERD plus E (G1) and BE (G2) samples were negative for Ki67 expression, while Ds (G3) and ADKs (G4) were positive although with a completely different staining pattern as compared to that displayed by hERG1. In fact, Ki67 positivity in Ds

TABLE 1. Comparison between *herg1* gene and hERG1 protein expression in different pathological conditions and statistical analysis in the clinico-pathological groups

	Positive samples/total (%)	Normal esophagus (*P*-value)	GERD (*P*-value)	GERD plus E (*P*-value)	Barrett's esophagus (*P*-value)
A: *herg1* expression evaluated through RT-PCR					
Normal esophagus	0/6 (0%)	/	0.317	0.118	0.008*
GERD	6/31 (19%)	0.317	/	0.166	0.002*
GERD plus E	13/40 (32%)	0.118	0.166	/	0.022*
Barrett's esophagus	9/13 (69%)	0.008*	0.002*	0.022*	/
B: hERG1 expression evaluated through IHC as reported in Materials and Methods					
Normal esophagus	0/6 (0%)	/	1.000	1.000	0.008*
GERD	0/31 (0%)	0.35	/	1.000	<0.0005*
GERD plus E	0/40 (0%)	1.000	1.000	/	<0.0005*
Barrett's esophagus	9/13 (69%)	0.008*	<0.0005*	<0.0005*	/

Numbers in the first column of both tables represent the number of positive cases on the total number of samples. The percentage of positive cases is reported in parentheses. Numbers in the other columns represent *P*-values obtained matching values referring to *herg1* gene (A) and hERG1 protein (B) expression in the different groups. Statistical analysis was performed through Fisher exact test.
*Statistically significant.

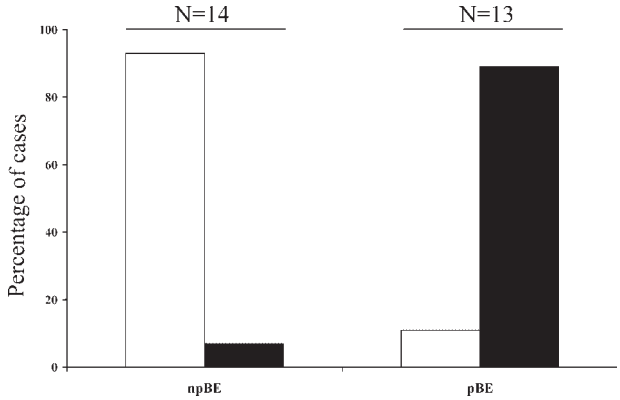

Fig. 4. hERG1 expression in BE samples in association with follow-up. Histogram relative to IHC data obtained on archival samples. Data are expressed as a percentage of the total number of samples examined. npBE, non progressed Barrett's esophagus (n = 14); pBE, progressed BE (n = 9). White bars, hERG1 negative samples; Black bars, hERG1 positive samples.

and ADKs was limited to sparse cells, while hERG1 protein was present in almost all dysplastic/cancerous epithelial cells.

Finally, we performed an IHC analysis on two cases which were sampled and followed from BE to the cancer stage, through D. IHC documentation of this evolution is reported in Figure 2, part H. It is evident that hERG1 expression in BE metaplastic cells (part H1) is maintained also in dysplastic (part H2) as well as true cancer cells (part H3).

Finally, BE samples where a 5-year or more follow-up was available were analyzed. Samples were subdivided into BE that did not proceed towards cancer (non-progressed BE, npBE) and BE that progressed towards adenocarcinoma (progressed BE, pBE) and the percentage of hERG1 positive samples at the IHC analysis was evaluated. It emerged (Fig. 4) that only 7% of the npBE showed hERG1 protein expression. On the other hand, almost all the pBE (89%) were hERG1 positive.

DISCUSSION

As far as we are aware, this article provides the first evidence of hERG1 potassium channel expression in physiologic and pathologic conditions of the human upper gastrointestinal tract. In particular, present findings show that hERG1 potassium channels are not expressed in normal squamous esophageal mucosa. Moreover, no hERG1 expression was detected in the gastroesophageal junction mucosa collected below the squamocolumnar junction, when the samples belonged to normal patients, or to patients with inflammatory conditions. On the contrary, hERG1 channels are mis- and overexpressed in the metaplastic mucosa present below the squamocolumnar junction that characterizes BE.

The discovery of hERG1 potassium channels in BE opens interesting biological perspectives. It has been proposed that metaplastic cells characterizing BE originate from a multipotential precursor cell present in the esophagus itself (Mueller et al., 2004). *herg1* may belong to the class of genes that are expressed in progenitor or true stem cells, to be actively transcribed as these cells start to proliferate in response to either growth factor/cytokine stimulation or neoplastic transformation. This hypothesis is supported by the fact that indeed hERG1 channels are expressed in stem cells present in a cancer cell population, and mark a peculiar stage of neoplastic cell differentiation (Biagiotti et al., 2006). Moreover, the *herg1* gene is not expressed in resting peripheral blood $CD34^+$ hematopoietic precursors, but its transcription is switched on when $CD34^+$ cells start to proliferate in response to growth factors and cytokines (Pillozzi et al., 2002). However, hERG1 expression does not configure just as a marker of proliferating cells. In fact, hERG1 immunostaining does not overlap with that of Ki67, a proliferation-related antigen that has been previously reported to be upregulated in BE and BE-derived ADKs (Fléjou, 2005). In particular, the expression of hERG1 protein in metaplastic cells characterizing BE is peculiar, since all BE samples turned out to be negative for Ki67 expression. In addition, Ki67 positivity in Ds and ADKs was limited to sparse cells, while hERG1 protein was present in almost all dysplastic/cancerous epithelial cells.

On the other hand, the *herg1* gene could be envisaged as a inflammation-related gene. In fact, we observed a discrepancy between RT-PCR (Reverse transcription and polymerase CHAIN reaction) and IHC data in E samples. This could be apparently traced back to a stronger presence of submucosal *herg1* positive tissues in those samples. Alternatively, a greater prevalence of inflammatory cells in samples from this groups could be responsible for the *herg1* gene expression. The latter hypothesis is confirmed by the fact that we indeed found a significant presence of inflammatory cells (witnessed by CD45 expression) in those endoscopic samples of the GERD plus E group that turned out to be positive for *herg1* expression at the PCR analysis.

In other words, while *herg1* is mainly expressed in inflammatory cells in inflammatory lesions of the lower esophagus, it is upregulated in epithelial cells in metaplastic lesions. The increase in *herg1* expression in inflammatory cells could be related to a cytokine-modulated effect of *herg1* transcription. To confirm our hypothesis, it has been recently reported that various cytokines (IL-4, IL-5, IL-9, and IL-13) as well as TNF-α can switch on cellular metaplasia in the airway epithelium. This occurs through the activation of the gene encoding a calcium-activated chloride conductance (CLCA1) that in turn determines transcription of mucin genes (Busse et al., 2005). The same role could be exerted by hERG1 channels in the upper gastrointestinal tract, and, hence, the hERG1 channel expression could be the determinant factor in the switching on of cellular metaplasia.

Another interesting result emerging from our data is the finding that the hERG1 protein is highly expressed in all the Ds as well as ADKs arising on a previously diagnosed BE. This fact suggests the hypothesis that *herg1* gene expression marks an early step along the progression of a normal cell toward a true cancerous cell through a metaplastic and dysplastic stage. Up to now, hERG1 channels have been found to be overexpressed only in truly cancerous conditions (Cherubini et al., 2000; Pillozzi et al., 2002; Smith et al., 2002; Lastraioli et al., 2004; Masi et al., 2005), while no hERG1 expression was detected in hyperproliferative conditions, such as endometrial hyperplasias or colorectal adenomas. In addition, hERG1 channels turned out to be more frequently expressed in advanced colorectal tumors. This may be due to the regulatory role exerted by hERG1 channels on the modulation of cell invasiveness (Lastraioli et al., 2004). In the upper gastrointestinal tract, this rule seems to be partially overwhelmed, since *herg1* expression is apparently switched

on in an early stage of the neoplastic pathway, that is, the metaplastic stage, to be further maintained throughout tumor progression. This different behavior could be related to different anatomical origin and the different physiological function of the metaplastic, goblet cells, that is, secretion. Indeed many types of K^+ channels, including hERG1 channels (Ashcroft, 2000), regulate cellular secretion in various types of cells. In addition, it is worth recalling that the onset of the metaplastic process in the upper gastrointestinal tract (esophagus, stomach) always strictly depends upon chronic inflammation. This process is apparently different from what happens in colorectal carcinogenesis, and could explain the early onset of aberrant expression of hERG1 channels. And in fact a similar early expression of hERG1 channels also occurs in gastric cancer progression (Lastraioli et al., unpublished data).

Finally, our findings of hERG1 expression marking a subset of patients whose BE lesion progressed to a true adenocarcinoma may also have a clinical impact. In fact, despite the limited number of patients examined, such finding suggests the possibility that patients susceptible to evolution to a true adenocarcinoma could be identified on the basis of hERG1 expression.

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LITERATURE CITED

Arcangeli A. 2005. Expression and role of hERG channels in cancer cells. In: Chadwick DJ, Goode J, editors. The hERG cardiac potassium channel: Structure, function, and long QT syndrome. Chichester, UK: Wiley, pp 225–234.

Arcangeli A, Becchetti A. 2005. Ion channels and cell proliferation. In: Janigro D, editor. Cell cycle in the central nervous system. Totowa, NJ: Humana Press, pp 81–94.

Ashcroft FM. 2000. Ion channels and disease. San Diego: Academic Press.

Biagiotti T, D'Amico M, Marzi I, Di Gennaro P, Arcangeli A, Wanke E, Olivotto M. 2006. Cell renewing in neuroblastoma: Electrophysiological and immunocytochemical characterization of stem cells and derivatives. Stem Cells 24(2):443–453.

Busse J, Zhang TF, Srivastava K, Lin BP, Schofield B, Sealfon SC, Li X-M. 2005. Chronic exposure to TNF-a increases airway mucus gene expression in vivo. J Allergy Clin Immunol 116:1256–1263.

Cherubini A, Taddei GL, Crociani O, Paglierani M, Buccoliero AM, Fontana L, Noci I, Borri P, Borrani E, Giachi M, Becchetti A, Rosati B, Wanke E, Olivotto M, Arcangeli A. 2000. HERG potassium channels are more frequently expressed in human endometrial cancers as compared to non-cancerous endometrium. Br J Cancer 83(12):1722–1729.

Fléjou JF. 2005. Barrett's esophagus: From metaplasia to dysplasia and cancer. Gut 54(Suppl 1):i6–i12.

Guillem PG. 2005. How to make a Barrett esophagus: Pathophysiology of columnar metaplasia of the esophagus. Dig Dis Sci 50(3):415–424.

Haggitt RC, Dean PJ. 1985. Adenocarcinoma in Barrett's epithelium. In: Spechler SJ, Goyal RK, editors. Barrett's esophagus. Pathophysiology, diagnosis, and management. New York: Elsevier Science, pp 153–166.

Jankowski JA, Wright NA, Meltzer SJ, Triadafilopoulos G, Geboes K, Casson AG, Kerr D, Young LS. 1999. Molecular evolution of the metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. Am J Pathol 154:965–973.

Lastraioli E, Guasti L, Crociani O, Polvani S, Hofmann G, Witchel H, Bencini L, Calistri M, Messerini L, Scatizzi M, Moretti R, Wanke E, Olivotto M, Mugnai G, Arcangeli A. 2004. *herg1* gene and HERG1 protein are overexpressed in colorectal cancers and regulate cell invasion of tumor cells. Cancer Res 64:606–611.

Masi A, Becchetti A, Restano-Cassulini R, Polvani S, Hofmann G, Buccoliero AM, Paglierani M, Pollo B, Taddei GL, Gallina P, DiLorenzo N, Franceschetti S, Wanke E, Arcangeli A. 2005. hERG1 channels are overexpressed in glioblastoma multiforme and modulate VEGF secretion in glioblastoma cell lines. Br J Cancer 93(7):781–792.

Merola E, Claudio PP, Giordano A. 2006. p53 and the malignant progression of Barrett's esophagus. J Cell Physiol 206(3):574–577.

Montgomery E, Goldblum JR, Greenson JK, Haber MM, Lamps LW, Lauwers GY, Lazenby AJ, Lewin DN, Robert ME, Washington K, Zahurak ML, Hart J. 2001. Dysplasia as a predictive marker for invasive carcinoma in Barrett esophagus: A follow-up study based on 138 cases from a diagnostic variability study. Hum Pathol 32(4):379–388.

Mueller J, Werner M, Stolte M. 2004. Barrett's esophagus: Histopathologic definitions and diagnostic criteria. World J Surg 28(2):148–154.

Newnham A, Quinn MJ, Babb P, Kang JY, Majeed A. 2003. Trends in oesophageal and gastric cancer incidence, mortality and survival in England and Wales 1971–1998/1999. Aliment Pharmacol Ther 17(5):655–664.

Ohya S, Asakura K, Muraki K, Watanabe M, Imaizumi Y. 2002. Molecular and functional characterization of ERG, KCNQ, and KCNE subtypes in rat stomach smooth muscle. Am J Physiol Gastrointest Liver Physiol 282(2):G277–G287.

Pillozzi S, Brizzi MF, Balzi M, Crociani O, Cherubini A, Guasti L, Bartolozzi B, Becchetti A, Wanke E, Bernabei PA, Olivotto M, Pegoraro L, Arcangeli A. 2002. HERG potassium channels are constitutively expressed in primary acute myeloid leukemias and regulate cell proliferation of normal and leukemic hemopoietic progenitors. Leukemia 16(9):1791–1798.

Sanguinetti MC, Tristani-Firouzi M. 2006. hERG potassium channel and cardiac arrhythmia. Nature 440(7083):463–469.

Sarzani R, Pietrucci F, Corinaldesi C, Francioni M, Letizia C, D'Erasmo E, Dessì-Fulgheri P, Rappelli A. 2006. The functional HERG variant 897 T is associated with Conn's adenoma. J Hypertens 24(3):479–487.

Smith GAM, Tsui HW, Newell EW, Jiang X, Zhu XP, Tsui FW, Schlicter LC. 2002. Functional up-regulation of HERG K^+ channels in neoplastic hemopoietic cells. J Biol Chem 277(21):18528–18534.

Spechler SJ. 2002. Clinical practice. Barrett's esophagus. N Engl J Med 346(11): 836–842.