6. Ion channels in cervical cancer: New perspectives in diagnosis and therapy

María de Guadalupe Chávez-López, Violeta Zúñiga-García and Javier Camacho

Department of Pharmacology, Centro de Investigación y Estudios Avanzados del IPN, Avenida Instituto Politécnico Nacional 2508, Mexico City 07360, Mexico

Abstract. Ion channels play crucial roles in most of the physiological processes including neural excitability, cardiac function, hormone secretion, and cell proliferation. In accordance, many ion channels are associated to human diseases and are targets of a huge amount of clinically used drugs. Recently, the participation of ion channels in tumor progression has gained great interest. Changes in ion channel expression are found in most malignancies including cervical cancer. For instance, anionic channels have been associated to cell cycle progression and malignant transformation of cervical cancer cells. In addition, voltage-gated sodium channels have been found in primary cultures from human cervical cancer, suggesting these channels as markers for this type of cancer. Voltage-gated potassium channels have been extensively studied in human cervical cancer, especially, members of the Eag (ether à-go-go) family. Eag1 channels display oncogenic properties and have been suggested as markers and therapeutic targets for cervical cancer. An interesting association between Eag1 protein expression and the grade of cervical intraepithelial neoplasia has been found, suggesting Eag1 as an early marker for cervical cancer. Accordingly, human papillomavirus
oncogenes and estrogens regulate Eag1 expression. Moreover, Eag1 channel blockers decrease proliferation of cells expressing HPV oncogenes. Human Erg channels have also been described in cervical cancer cell lines. Despite further studies are needed in order to determine the molecular mechanisms by which ion channels influence tumor progression, these proteins are arising as new promising tools for cervical cancer diagnosis and therapy.

Cervical cancer is the most common gynecological malignancy worldwide. This disease is strongly associated with infection by oncogenic types of human papillomavirus (HPV), but only a small fraction of patients develop cancer, indicating that other factors like estrogens contribute to cervical cancer progression (1). Ion channels are membrane proteins that allow the passage of ions and play pivotal roles in neuronal and muscle excitability, hormone secretion, cell proliferation and cardiac contraction (2). In the last decades, these proteins have gained great interest in oncology, including cervical cancer. The expression and/or activity of different ion channels regulate specific stages of cancer progression. Their contribution to the neoplastic phenotype includes the control of cell proliferation and apoptosis, as well as the regulation of invasiveness and metastatic spread. Interestingly, some of these roles can be attributed to signaling mechanisms independent of ion flow (3). In this chapter, we will describe first some basic properties of ion channels. Then we will offer a general overview of ion channels in several diseases including cancer. Finally, we will focus on the expression of ion channels in cervical cancer, and their potential role as markers and therapeutic targets for this disease.

**Ion channels**

Ion channels are membrane proteins (Figure 1) that control ion flux, and therefore the electrochemical gradient across the membrane of every living cell (4). These channels act as gates that are closed or opened according to external stimuli and are able to discriminate between ions passing through them, so in most cases they are selective to a particular ion. These proteins are involved in a wide range of physiological processes such as nerve and muscle excitation, sensory transduction, cell proliferation and metabolism, among others (2).

Ion flow rate at up to $10^6$ ions per second, provides a very efficient cellular ion transport. This movement is due to electrochemical gradient (5). Depending on the stimulus that causes channel opening, they are classified into different types including: voltage-gated channels, ligand-gated channels, mechanosensitive channels and activated by temperature channels (2).

In addition, ion channels may be regulated by calcium, pH, phosphorylation and lipids (6). In the case of voltage-gated ion channels, these might be in one of at least three different conformations: open (conducting), or in one of the non-conducting states, namely, closed, or inactivated (Figure 2).
Figure 1. Ion Channels. These proteins cross the cell membrane, thus, having extracellular, intracellular and transmembrane domains.

Figure 2. Conformational states of voltage-gated ion channels. Channels might be open upon membrane depolarization. Several types of voltage-gated ion channels become then inactivated. Other channels return to the closed state upon hyperpolarization.

Voltage-gated channels display a voltage-sensor formed by several charged amino acids that move in the electric field of the membrane upon depolarization resulting in channel opening. In the case of ligand-gated channels, the corresponding sensor is a region of the channel that is exposed either outside or inside the membrane, and binds specific molecules with high affinity leading to channel opening. Mechano-sensitive channels, like
those found in the Pacinian corpuscles, are opened by stretching the cell membrane in response to either pressure or tension (5).

Channels reside not only in the plasma membrane, but also in membranes of intracellular organelles. Ion channels participate in many functions including neuronal transmission, transepithelial transport of salt and water important for cell volume and pH regulation, hormonal secretion, muscle contraction, cell migration, cell cycle progression, apoptosis and gene transcription. Thus, many human diseases are closely associated to alterations in either ion channel function or expression (6).

**Channelopathies**

Since the identification in 1989 of the first disease associated with an ion channel, namely, cystic fibrosis, the list of diseases associated to ion channels is still growing. The concept of *channelopathy* refers to defects in the function of ion channels that lead to significant physiological changes in various tissues (5, 6).

Channelopathies can be produced by different mechanisms including the genetic and autoimmune diseases. Among the genetic alterations are mutations that may cause either loss or gain of channel function. Loss-of-function mutations in ion channels often lead to recessive disease, as is the case with CFTR (Cystic Fibrosis Transmembrane Regulator) mutations or with CLCNKB mutations in Bartter syndrome. However, as many ion channels are multimeric complexes, certain loss-of-function mutations may give rise to dominant-negative mutants. These can reduce channel function below the 50% level expected from non-interacting proteins in a heterozygous patient. Whilst such dominant-negative effects can reduce channel function down to one-quarter of wild-type with dimeric channels, the effect can be much stronger (reduction up to one-sixteenth) with tetrameric channels. Depending on the presence or absence of dominant negative effects, mutations in the same gene may result in recessive or dominant disease, as a general rule, patients with recessive mutations are more severely affected than patients with dominant mutations. Gain-of-function mutations are most often associated with dominant inheritance of the disease. Mutations in various isoforms of voltage-dependent Na\(^+\) channels cause paramyotonia, cardiac arrhythmia and epilepsy because they result in additional, late Na\(^+\) currents due to defective inactivation. Mutations in the epithelial Na\(^+\) channel ENaC that result in an increased plasma membrane expression cause dominant hypertension in Liddle syndrome (5, 6).
Among the genetic alterations there are also mutations in the promoter region of the gene encoding an ion channel. This can cause either sub-expression or overexpression of the channel protein, resulting in changes in the channel number and the corresponding change in function. The third type of genetic alteration that determines channel dysfunction are mutations in genes encoding regulatory molecules of ion channels either by defects in its structure by themselves, or by defects in the pathways leading to their production (5, 6).

A huge variety of diseases associated with ion channels can be found. In skeletal muscle, mutations in Na\(^+\), K\(^+\), Ca\(^{2+}\) and voltage-activated Cl\(^-\) channels and acetylcholine receptors lead to disorders such as hyper- and hypokalemic paralysis, myotonia, malignant hyperthermia and myasthenia. In the nervous system alterations in Na\(^+\), K\(^+\) and Ca\(^{2+}\) voltage-gated channels, as well as in either the acetylcholine, dopamine, or GABA receptor, could explain several pathologies including epilepsy, episodic ataxia, familial hemiplegic migraine, the Lambert-Eaton syndrome, Alzheimer disease, Parkinson's disease, schizophrenia, and hyperreflexia. Some kidney diseases like Bartter's syndrome, polycystic kidney disease and Dent's disease, and endocrine problems such as hyperinsulinemia and hypoglycemia of infancy are linked to mutations in ion channels. Some vision disorders such as congenital stationary night blindness and total color blindness may be linked to mutations in ion channels. In the heart, it has been proposed that mutations in either Na\(^+\) or K\(^+\) channels lead to three major disorders: the long QT syndrome, Brugada syndrome and disease of the conduction system (5, 6). Because of their role in health and disease, ion channels are the targets of a huge amount of clinically used drugs. Some examples of channelopathies are listed in Table 1.

Cancer is a multifactorial disease and several ion channels are involved in tumor cell proliferation. On the other hand both voltage- and ligand-gated channels have been involved in the control of different cell cycle checkpoints. Cell proliferation involves at some point the activation of Cl\(^-\), K\(^+\) and Ca\(^{2+}\) channels. Thus, alterations in either channel expression or activity may be responsible for the development and growth of cancer cells.

The EAG potassium channel (7), and the sodium channels Na\(_v\) 1.2 and 1.7 channels (8) are some examples of the channels implicated in the cervical cancer. Before going into details of ion channels in cervical cancer, next, we will describe the expression and participation of ion channels in tumor cell proliferation and migration.
Table 1. Some diseases associated to alterations in ion channels (6, 8, 28, 29).

<table>
<thead>
<tr>
<th>CHANNEL</th>
<th>GENE</th>
<th>DISEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sodium Channels:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na\textsubscript{1.1}</td>
<td>SCN1A</td>
<td>Generalized epilepsy with febrile seizures (GEFS+)</td>
</tr>
<tr>
<td>Na\textsubscript{1.2}</td>
<td>SCN2A</td>
<td>Generalized epilepsy and cervical cancer</td>
</tr>
<tr>
<td>Na\textsubscript{1.4}</td>
<td>SCN4A</td>
<td>Paramyotonia congenital, potassium aggressive myotonia, hyperkalemic periodic paralysis</td>
</tr>
<tr>
<td>Na\textsubscript{1.5}</td>
<td>SCN5A</td>
<td>Long-QT syndrome, progressive familial heart block type 1, Brugada syndrome</td>
</tr>
<tr>
<td>Na\textsubscript{1.7}</td>
<td>SCN7A</td>
<td>Cervical cancer</td>
</tr>
<tr>
<td><strong>Potassium Channels:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K\textsubscript{1.1}</td>
<td>KCNA1</td>
<td>Episodic ataxia with myokymia and breast cancer</td>
</tr>
<tr>
<td>K\textsubscript{1.3}</td>
<td>KCNA3</td>
<td>Breast, prostate and pancreas cancer</td>
</tr>
<tr>
<td>KCNQ2</td>
<td>KCNQ2</td>
<td>BFNC (epilepsy) with myokymia</td>
</tr>
<tr>
<td>KCNQ3</td>
<td>KCNQ3</td>
<td>BFNC (epilepsy)</td>
</tr>
<tr>
<td>KCNQ4</td>
<td>KCNQ4</td>
<td>DFNA2 (dominant hearing loss)</td>
</tr>
<tr>
<td>HERG</td>
<td>KCNH2</td>
<td>Long-QT syndrome</td>
</tr>
<tr>
<td>hEag1</td>
<td>KCNH1</td>
<td>Several types of cancer like cervical, breast, colon, prostate, HCC and others.</td>
</tr>
<tr>
<td>Kir1.1</td>
<td>KCNJ1</td>
<td>Bartter syndrome (renal salt loss, hypokalemic alkalosis)</td>
</tr>
<tr>
<td>Kir2.1</td>
<td>KCNJ2</td>
<td>Long-QT syndrome with dysmorphic features</td>
</tr>
<tr>
<td>Kir6.2/K\textsubscript{ATP}</td>
<td>KCNJ11</td>
<td>Persistent hyperinsulinemic hypoglycemia of infancy (PHHI)</td>
</tr>
<tr>
<td><strong>Chloride Channels:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFTR</td>
<td>ABCC7</td>
<td>Cystic fibrosis, congenital bilateral aplasia of vas deferens</td>
</tr>
<tr>
<td>CIC-1</td>
<td>CLCN1</td>
<td>Autosomal recessive or dominant myotonia</td>
</tr>
<tr>
<td>CIC-5</td>
<td>CLCN5</td>
<td>X-linked proteinuria and kidney stones</td>
</tr>
<tr>
<td>CIC-7</td>
<td>CLCN7</td>
<td>Osteopetrosis</td>
</tr>
<tr>
<td>CIC-Kb</td>
<td>CLCNKB</td>
<td>Bartter syndrome type III</td>
</tr>
<tr>
<td><strong>Calcium Channels:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca\textsubscript{1.1}</td>
<td>CACNA1S</td>
<td>Hypokalemic periodic paralysis, malignant hyperthermia</td>
</tr>
<tr>
<td>Ca\textsubscript{1.4}</td>
<td>CACNA1F</td>
<td>X-linked congenital stationary night blindness</td>
</tr>
<tr>
<td>Ca\textsubscript{2.1}</td>
<td>CACNA1A</td>
<td>Familial hemiplegic migraine, episodic ataxia, spinocerebellar ataxia type 6</td>
</tr>
</tbody>
</table>

**Participation of ion channels in tumor cell proliferation and tumor progression**

Cell homeostasis requires a delicate balance between formation of new cells by cell proliferation and their elimination by apoptosis. Cell proliferation is stimulated by growth factors (9). Cell proliferation involves the activation of Cl\textsuperscript{−}, K\textsuperscript{+} and Ca\textsuperscript{2+} channels. Because the respective channel inhibitors have been
reported to interfere with proliferation, the channels appear to play an active role in the machinery leading to duplication of a given cell (9).

**Ion channels and the cell cycle**

The cell mitotic cycle comprises a sequence of events that must be precisely coordinated, such as DNA replication, chromosome condensation and segregation, duplication and migration of the spindle pole, breakdown of the nuclear envelope and cytokinesis (10). Proper progress through the cell cycle is ensured by checkpoint controls that monitor DNA integrity and the completion of each molecular event before allowing transition to the next phase (10). In eukaryotic cells, the main checkpoints are placed at the G/S transition, in late S (DNA synthesis) phase, at mitosis (M) entry, and at the metaphase-to-anaphase transition (10) (Fig 3). Expression of ion channels changes during the cell cycle and many of them have been suggested to have a role in the progression of particular phases (Fig. 3). Some other channels might have a general role in the cell cycle. Following, some potential mechanisms suggesting how ion channels participate in cell cycle progression are discussed.

![Figure 3. Ion channels and the cell cycle checkpoints. Schematic relation between ion channel expression and the main cell cycle checkpoints. Several ion channels have been suggested to have a role at the different phases of the cell cycle.](image-url)
General mechanisms

In principle, ion channels may affect proliferation in two different ways: any cell requires ion channel function to maintain basic homeostasis parameters, such as intracellular \( \text{Ca}^{2+} \), pH and cell volume, and to allow uptake of substrates and release of metabolic products. Thus, inhibition of those channels will lower cell proliferation without interfering at a particular step during the cell cycle (11). On the other hand, ion channel activity, such as the hyperpolarizing activity of \( \text{K}^+ \) channels, is required at special checkpoints during the cell cycle and therefore will have a precise role in proliferation (11).

Numerous reports have shown that the activity of \( \text{K}^+ \), \( \text{Ca}^{2+} \) and \( \text{Cl}^- \) channels changes during cell cycling and suggest that this channel oscillation itself may be either a result of cell cycling or may be part of the clock mechanism (11). Early evidence suggested that the increase in \( \text{K}^+ \) channel expression and activity at the G1/S boundary is often necessary for cells to progress through the cell cycle (10). In addition, the inhibition of \( \text{K}^+ \) currents and the consequent membrane depolarization cause accumulation of the cyclin-dependent kinase inhibitors p27 and p21. Thus, cell cycle-relevant proteins may be also directly regulated by membrane voltage (11).

Modulation of \( \text{Ca}^{2+} \) entry

Calcium ions regulate various cellular processes by activating or inhibiting cellular signalling pathways and \( \text{Ca}^{2+} \)-regulated proteins. These processes range from muscle contraction to synaptic transmission and from cellular proliferation to apoptosis (13). \( \text{Ca}^{2+} \) is highly regulated within cellular compartments (12) and released upon mitogenic stimulation (11). There are three major classes of membrane-associated proteins directly involved in \( \text{Ca}^{2+} \) homeostasis: channels, ATPases (pumps) and exchangers (13). In excitable tissues, \( \text{Ca}^{2+} \) influx occurs through voltage-gated \( \text{Ca}^{2+} \) channels. In non-excitable tissues hyperpolarization of the membrane voltage is important for the increase in intracellular \( \text{Ca}^{2+} \), providing the driving force for \( \text{Ca}^{2+} \) entry from extracellular space (11). Some \( \text{Ca}^{2+} \)-mediated signalling pathways are involved in tumorigenesis and tumor progression, such as metastasis, invasion and angiogenesis. (13). Calcium entry through \( \text{Ca}^{2+} \) channels and subsequent intracellular \( \text{Ca}^{2+} \) mobilization favors tumor cell growth (14). This notion is in line with the fact that verapamil, a \( \text{Ca}^{2+} \) channel antagonist, inhibits cell proliferation in several tumor cell lines including human small-cell carcinoma of the lung, and H35 hepatocarcinoma cells (14).
Transmembrane potential

Transmembrane potential \((V_m)\) refers to the voltage difference across a cell’s bilayer membrane that is established by the balance of intracellular and extracellular ionic concentration. This balance is maintained via passive and active ion transport through various ion channels and transporters located within the membrane. Several evidences support the functional role of \(V_m\) in the regulation of proliferation and differentiation (15). In general, cancer cells display more positive transmembrane potentials than healthy cells of the same histological origin (14). The change in average \(V_m\) between dividing and quiescent cell populations often depends on a shift in the cellular complement of ion channels (16). Membrane depolarization has been believed to be the key to unlimited tumor cell proliferation, presumably due to facilitation of \(Ca^{2+}\) entry through activation of voltage-dependent \(Ca^{2+}\) channels at less negative voltages (14). The ability to control cell functions by modulating bioelectric properties such as \(V_m\) would be an invaluable tool for directing stem cell behavior toward therapeutic goals (15).

Non-conducting roles of ion channels

Recent works has demonstrated that many ion channels can themselves directly influence biochemical events by mechanisms that do not directly depend on their function as ion channels. Such control over cellular signalling has been reported for each of the major classes of ion channels that influence excitability, including the sodium, calcium and potassium channels that shape action potentials, and the non-selective cation channels that control resting membrane properties (3). For instance, voltage-dependent ion channels in neurons do more than regulate firing patterns. First, either deletion or overexpression of channels alters the properties of a cell in ways that cannot be readily explained solely by effects on excitability, and, in a subset of these channels, mutant non-conducting subunits lacking a functional pore have the same effects as the functional channel (3). The possible modulation of intracellular pathways by enzymatic roles of channel proteins or conformational coupling with other proteins is converging onto the transcriptional regulation of cancer-related genes (16). Recent findings have shown that a number of ion channel subunits demonstrate biological activity that is unrelated to ion conduction (Table 2) (3).

Particularly interesting evidence concerns \(K_v10.1\) channel, that is normally expressed in the central nervous system but can be overexpressed in human cancers (10). In murine fibroblasts transfected with \(K_v10.1\), proliferation is stimulated in a way that depends not on ion flow, but on the
voltage sensor conformation. For example, cell transfection with either wild type or non-conducting mutant channels produces a similar stimulation of proliferation (reviewed in ref. 16).

Other experiments showed that the gating of Kv10.1 is directly linked to several intracellular messenger pathways related to the mitotic control, which include the p38, MAPK, but not ERK proteins (10). Thus, non-conducting functions of ion channels offer alternative explanations on the molecular mechanisms by which ion channels participate in tumor cell proliferation.

Table 2. Some non-conducting functions of channel α- and β-subunits. (Based on Kaczmarek 2006).

<table>
<thead>
<tr>
<th>Ion channels</th>
<th>Biological activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TRP channels</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRPM6</td>
<td>Protein kinase</td>
<td>Shlingmann, K.P., et al. 2002 (38); Shmitz, C., et al. 2005 (39)</td>
</tr>
<tr>
<td>TRPM7</td>
<td>Protein kinase</td>
<td>Runnels, L. W., et al. 2001(40)</td>
</tr>
<tr>
<td><strong>Ca&lt;sup&gt;2+&lt;/sup&gt; channels</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cav1.1</td>
<td>Conformational coupling to ryanodine receptors</td>
<td>Rios, E., et al. 1991(41)</td>
</tr>
<tr>
<td>Cav2.1</td>
<td>Linkage to SNARE proteins</td>
<td>Mochida, S., et al.1998 (42)</td>
</tr>
<tr>
<td><strong>K&lt;sup&gt;+&lt;/sup&gt; channels</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAG</td>
<td>Activation of CaMKII Eag1 contributes to tumor progression independently of its primary function as an ion channel</td>
<td>Sun, X., X., et al. 2004 (43); Griffith, L. C. 2004 (44) Downie, B., et al. 2008 (58)</td>
</tr>
<tr>
<td><strong>K&lt;sup&gt;+&lt;/sup&gt; channel β-subunits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k&lt;sub&gt;A&lt;/sub&gt;B&lt;sub&gt;1&lt;/sub&gt;, K&lt;sub&gt;A&lt;/sub&gt;B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Aldo reductases</td>
<td>Weng, J., et al. 2006 (45)</td>
</tr>
<tr>
<td>KChIP1-4</td>
<td>Transcription factors, regulators of presenilin</td>
<td>Buxbaum, J. D., et al. 1998 (46); Carrion, A. M., et al. 1999 (47); Buxbaum, J. D., 2004 (48); Savignac, M., et al. 2005 (49)</td>
</tr>
<tr>
<td><strong>Na&lt;sup&gt;+&lt;/sup&gt; channel β-subunit</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β1-β4</td>
<td>Homophilic and heterophilic cell adhesion</td>
<td>McEwen, D. P., et al. 2004 (50); Davis, T. H., et al. 2004 (51)</td>
</tr>
<tr>
<td><strong>Ca&lt;sup&gt;2+&lt;/sup&gt; channel β-subunit</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calmodulin</td>
<td>Activation of MAP kinase</td>
<td>Dolmetsch, R. E., et al. 2001 (52)</td>
</tr>
</tbody>
</table>
A role for ion channels in cancer cell migration

Cell migration plays an integral role in several normal physiological processes, including neural crest cell migration, leukocyte extravasation from the vasculature, and fibroblast migration during wound healing. Cell migration is also critical to cancer metastasis and malignant progression. Migrating cells are polarized and move along a front-to-back axis (17).

A growing body of evidence indicates that malignant cells hijack physiological mechanisms for cell migration, especially the use of ion channels to promote motility. Malignant cells can commandeer physiological mechanisms for migration to increase disease spread. Specifically, malignant cells over-express a variety of K⁺, Cl⁻, and Na⁺ channels that use the ionic gradients created by NKCC1 (an active co-transporter that brings Na⁺, K⁺, and 2 Cl⁻ into the cell) and facilitate motility and invasion. Several preliminary studies demonstrating that pharmacological inhibition of ion channels decreases malignant motility led investigators to hypothesize that ion channels facilitate the migration of transformed cells. The molecular identities of many of these channels were subsequently discerned through knockdown studies and have been shown that ion channels and transporters, while facilitating motility in non-malignant cells, are also intimately involved in the migration of neoplastic cells by dynamic volume regulation (17). Therefore, ion channel expression might favor cancer progression by either stimulating proliferation or inducing migration. Finally, we will focus on the expression of ion channels and its potential role in cervical cancer.

Cervical cancer

Carcinomas of the anogenital tract (particularly cancer of cervix) account for almost 12% of all cancers in women, and represent the second most frequent gynecological malignancy in the world (1).

Cervical cancer remains a major worldwide health concern. This occurs despite the use of Pap smears, a highly successful technique for early detection of cervical cancer precursor cells but limited in its use to countries with highly developed health care systems. Current approaches for treating cancer have limited success; consequently, 5-year survival rates for women with cervical cancer remain low. It is estimated that 500,000 women annually will develop cervical cancer; 200,000 women die every year from this malignancy (1, 18).

The high risk human papillomavirus (HPVs) types, such as HPV 16 and 18 are associated with >99% of cervical cancers and are considered to be the
major etiologic factor in this and other anogenital cancers as well as a significant portion of head and neck cancers within the oral cavity (18). The papillomavirus cycle differs from all other virus families: infection requires the availability of epidermal or mucosal epithelial cells that are still able to proliferate (basal layer cells). Several oncoproteins are found in HPV including E6 and E7 (Figure 4). Following entry into the suprabasal layers, late viral gene expression is initiated; the circular viral genome is then replicated and structural proteins form. Complete viral particles are assembled and released in the upper layers of the epidermis or mucosa (1).

A more significant role for malignant transformation can be assigned to the E6 and E7 genes and their respective proteins. They are consistently expressed in malignant tissue, and inhibiting their expression blocks the malignant phenotype of cervical cancer cells. They are independently able to immortalize various human cell types in tissue culture, but efficiency is increased when they are expressed together. E6 interacts with p53, and E7 interacts with RB (retinoblastoma protein) to block the activity of these tumor suppressors. Indeed, some of the prominent functions of the E6 protein originate from its interaction with, followed by degradation of, p53 and the pro-apoptotic protein BAK. E7, however, interacts with and degrades RB, which releases the transcription factor E2F from RB inhibition (1).

Figure 4. HPV DNA organization. E6 and E7 oncoproteins target the tumor suppressor proteins p53 and retinoblastoma.
The development of cervical cancer is a multifactorial process and likely involves other contributing factors in addition to HPVs, such as environmental, genetic, biological, and hormonal factors. A role of estrogens in human cervical cancer has been hypothesized on the basis of two observations. First, extended use of oral contraceptives, which contain synthetic estrogens and/or progesterone, increases cervical cancer risk 2- to 4-fold, depending upon the length of use. The synthetic estrogens found in oral contraceptive formulations have increased estrogenic activity compared with endogenous estrogen in some tissues, as well as enhanced bioavailability. Second, parity increases cervical cancer risk up to 3.8-fold for seven or more pregnancies (18). During pregnancy, women are exposed to continuously increased levels of estrogen. There are also studies showing that transgenic E7- mice treated with estrogens develop cervical cancer (18). Estrogen receptors ERα and ERβ belong to the family of nuclear receptors that function as transcription factors, thereby promoting cancer cell proliferation. ERα and ERβ can have opposite actions, suggesting that the proliferative response to estradiol in cervix is the result of a balance between ERα and ERβ signaling (19).

Several ion channels are expressed in cervical cancer cells and seem to play different roles from regulating cell volume to favor invasiveness. Following we will describe the expression and potential roles of ion channels in cervical cancer.

**Ion channels as markers and therapeutic targets of cervical cancer**

**Anion channels**

Chloride channels are essential for the transport of salt and water across the membrane in many epithelial cells. Three distinct chloride currents, regulated by cAMP, Ca^{2+}, and cell volume have been demonstrated in airway epithelial cells and in the T84 colonic carcinoma cell line (20). Volume regulation is a widespread process that enables cells to maintain their normal volume despite changes in extracellular osmolarity (20). Epithelial cells possess multiple volume-sensitive transport pathways leading to regulatory volume decrease (RVD) in response to hypotonic stress. During cell cycle progression, cells undergo a significant increase in size (especially at the G_1/S transition), which perturbs cell volume homeostasis and is counterbalanced by RVD (21).

The signaling of cell proliferation may require transient cell shrinkage at some stage, which may be accomplished by activation of Cl^- channels (22).
As intracellular Cl⁻ activity is usually above electrochemical equilibrium, activation of Cl⁻ channels leads to Cl⁻ efflux and thus depolarization. If K⁺ channels are simultaneously active, the Cl⁻ efflux is paralleled by the movement of K⁺. The loss of KCl and osmotically obliged water then leads to cell shrinkage. In ras oncogene-expressing cells, cell shrinkage is required for the initiation of cytosolic Ca²⁺ oscillations, which are in turn needed for the stimulation of cell proliferation (23).

In the case of cervical cancer, the membrane transport properties of human cervical epithelial cells have received little attention (21). Activation of volume-activated chloride currents is associated with malignant transformation of human cervical squamous epithelium, independent of its association with HPV (20). In addition, it has been shown that KCl and organic osmolyte efflux are strongly upregulated during human cervical carcinogenesis (21). Co-activation of K⁺ channels and VRACs (volume-regulated anion channels) is therefore proposed to be a necessary step for volume regulation during cell cycle progression. Accordingly, the pharmacological blockade of VRACs by tamoxifen or NPPB caused a dose-dependent inhibition of proliferation of cervical cancer cells (21).

Voltage-gated Na⁺ channels

Voltage-gated sodium channels (VGSC or Naᵥ) have been characterized in excitable cells including nerve, muscle and neuroendocrine cell types, and they are also expressed in “non excitable” cell types, such as lymphocytes, osteoblasts, endothelial, fibroblasts and renal tubule epithelial cells (24, 8). These channels consist of one α subunit of ~260 kDa, with four domains, and two associated β subunits. Each α subunit has six transmembrane spans; the fourth transmembrane span contains the voltage sensor of the channel. VGSCs have been shown to play a role in cell proliferation, migration, adhesion, neural conduction, and muscle contraction (24). Sodium influx via VGSCs originates the rising phase of neuronal action potentials (2, 24).

Ten α subunits and four β subunits for VGSCs are known. The α subunits appear to have tissue specific expression profiles (24). As is the case for K⁺ channels, VGSCs are dysregulated in a variety of tumors, including breast, lung -both small-cell and non-small-cell and prostate cancers, leukemia and, more recently, cervical cancer and mesothelioma (25). Pharmacological inhibition of VGSCs decreases proliferation in such cells (24).

In addition, VGSCs increase the metastatic potential of cancer cells, although the mechanisms involved are not well understood (25). Enhanced metastasis correlates with the appearance of membrane channels and
ions that are characteristic of excitable membranes. The upregulated expression of VGSCs has been detected in biopsies of metastatic breast, prostate and cervical carcinomas as well as in highly metastatic cancer-derived cell lines. Interestingly, it seems that the presence of the \(\text{Na}^+\) current rather than the excess of a specific VGSC protein is crucial for metastasis because in highly metastatic breast cancer cells the \(\text{Na}_v1.5\) channel was found to be \(\sim 1000\)-fold overexpressed, whereas in metastatic PCa tissues the \(\text{Na}_v1.7\) channel was upregulated \(\sim 20\)-fold (26). On the other hand, \(\text{Na}_v1.5\) activity increased the invasiveness of human breast cancer cells through increased cysteine cathepsin activity. Cysteine cathepsins are proteolytic enzymes with many roles in cancer metastasis (25).

In human cervical cancer cells, the presence of \(\text{Na}_v1.2\), \(\text{Na}_v1.4\), \(\text{Na}_v1.6\) and \(\text{Na}_v1.7\) channels has been reported in primary cultures obtained from human cervical cancer biopsies of patients who had not received any anticancer therapy (8). Notably, in tissue from normal cervix biopsies, only the expression of \(\text{Na}_v1.4\) was detected (8). Interestingly, \(\text{Nav}1.6\) channels have been associated to the invasiveness of cervical cancer cells. In accordance, channel inhibition decrease the invasive properties of cervical tumor cells (57).

**Voltage-gated K\(^+\) channels**

Voltage-gated potassium channels (VGKC or \(\text{K}_v\)) are found in both excitable and non-excitable cells and are the most diverse family among the voltage-gated ion channels (24). There are more than 100 genes that encode subunits of \(\text{K}^+\) channels. These channels are responsible for maintaining the membrane resting potential, they also determine the shape of the action potentials in several cells and regulate firing patterns such as pacing or bursting (2). All \(\alpha\) subunits of \(\text{K}_v\) channels share a similar organization, with each polypeptide containing six putative transmembrane segments (S1-S6), a voltage sensor (S4), a pore region between the segments S5 and S6 and N- and C-terminal domains of varying lengths, both localized at the cytoplasmic side of the membrane (27). Functional \(\text{K}_v\) channels are formed by symmetric arrangement of four \(\alpha\) subunits surrounding a central water-filled pore that is highly selective for \(\text{K}^+\) ions (27).

\(\text{K}^+\) channels can be divided into four structural types based on their mode of activation and the number of their transmembrane segments: inwardly rectifying 2-transmembrane \(\text{K}^+\) channels (\(\text{K}_{i1}\)), 2-pore 4-transmembrane \(\text{K}^+\) channels (\(\text{K}_{2p}\)), \(\text{Ca}^{2+}\)-activated 6-transmembrane or 7-transmembrane \(\text{K}^+\) channels (\(\text{K}_{\text{Ca}}\)), and voltage-gated 6-transmembrane \(\text{K}^+\) channels (\(\text{K}_v\)) (28).
It is well known that K^+ channels take part in a number of processes such as differentiation, cell volume regulation, activation and apoptosis (12). As a crucial cellular function, cell proliferation is very strictly controlled by several independent mechanisms. Evidence has accumulated pointing to K^+ channels as relevant players in the control of this process (22) by modulating calcium influx, membrane potential and cell volume (12).

K^+ channel activity is altered during cell cycle progression. Hyperpolarization due to K^+ channel activation is required for the initiation of the G1 phase of the cell cycle (26). The activation of K^+ channels leads to K^+ efflux which generates the required transient hyperpolarization. A rational explanation would be that K^+ channel are needed to control a specific check point in the G1/S transition. Accordingly, K^+ channels exhibit a cell-cycle-dependent expression, being transiently up-regulated during the G1 or S phase (12). The inhibition of K^+ channel function leads to a decreased in proliferation both in models in which proliferation is a physiological response (the case of lymphocytes) and in those in which it is a manifestation of a pathological condition (as in cancer cells) (22).

Various studies have shown that K^+ channels are also involved in cancer progression and many tumors show impaired expression (25). In some cases, the expression of K^+ channels correlates with the degree of tumorigenicity, such as for Eag1 (Kv10.1), TASK3 (K2p9.1), and HERG (Kv11.1) channels (12).

Among the various types of K^+ channels, Ether à go-go (Eag1) was first described as a cell-cycle regulated channel relevant in the process of myoblast fusion and has gained great interest in cervical cancer diagnosis and therapy (31). Human Eag1 (hEag1) mRNA shows restricted distribution in healthy tissues, it is expressed mainly in brain, and slightly in placenta, testis, and adrenal gland. Interestingly, Eag1 is abundantly expressed in tumor cells, including those from cervical, lung, breast, colon, and prostate cancer; therefore, Eag1 is suggested as a cancer marker (28-30). This restricted distribution in normal tissues is one of the most attractive features of Eag1 as a potential tumor marker.

In recent years, the role of Eag1 in the control of cell proliferation was explored and found that it shows both transforming properties in vitro (i.e. it confers loss of contact inhibition and increased growth rate) and increases the speed of growth and the invasiveness of tumours implanted into SCID mice in vivo (22, 29, 33). The specific inhibition of Eag1 expression by antisense technology, siRNA, specific monoclonal antibodies or by non-specific blockers leads to a reduction of tumour cell proliferation in vitro and/or in vivo (28-30, 33-35). Thus, Eag1 is also suggested as a cancer therapeutic target (30).
Eag1 expression has been found in 100% of cervical cancer samples and in 33% of the normal biopsies (7), these normal cervical samples came from women with negative pap smears, including a patient with HPV infection. These observations suggested Eag1 expression as an early sign of cellular hyperproliferation (30). \( K_v11 \) channels (also known as ERG, from \textit{Ether á go-go} Related Gene, \textit{hERG} in humans) have also been associated to tumor cell proliferation. Three different genes constitute the \( K_v11 \) subfamily in

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
\textbf{CHANNEL} & \textbf{FUNCTION} & \textbf{NORMAL TISSUE} & \textbf{CERVICAL CANCER} & \textbf{REF.} \\
\hline
VRACs (volume-regulated anion channels) & Participate in several physiological processes, such as osmolyte transport, metabolism, hormone release, cell migration, proliferation and differentiation. & VRACs are ubiquitously expressed in virtually all mammalian cells. & VRACs channel are significantly activated in cervical cancer cells such as SiHa, HT-3, CasKi, CX. Tamoixfen inhibits proliferation of cervical cancer cells. & Nilius et al. 1996 (53); Shen et al. 2000 (21); Lang et al. 1998 (54). \\
\hline
Voltage-Gated \( \text{Na}^+ \) Channels (\( \text{Na}_v \)) & Na\(_v\) are expressed in excitable cells including nerve, muscle and neuroendocrine cells, and are also expressed in non excitable cell types such as lymphocytes, osteoblasts, and renal tubule epithelial cells. & Na\(_v1.2\), Na\(_v1.4\), Na\(_v1.6\) and Na\(_v1.7\) channels are expressed in primary cultures obtained from human cervical cancer biopsies. Nav1.6 channels are associated to invasiveness of cervical cancer cells. & \begin{itemize}
\item Marban et al. 1998 (55);
\item Fiske et al. 2006 (24);
\item Diaz 2007 (8);
\item Hernandez-Plata E. 2012 (57)
\end{itemize} \\
\hline
Voltage-Gated \( \text{K}^+ \) Channels (\( \text{K}_v \)) & A major function of \( \text{K}^+ \) channels is to set the membrane potential and thereby regulate the electrical excitability of the cell. & Kv10.1 (Eag1) is found in cervical cancer biopsies and in the cervical cancer cell lines HeLa, SiHa, C33, and Caski. Eag1 channels are up-regulated by estrogens and HPV. HERG mRNA is also expressed in HeLa cells. & \begin{itemize}
\item Diaz 2009 (30);
\item Hille 2001 (2);
\item Stühmer, 1989 (56);
\item Pardo 2004 (22).
\end{itemize} \\
\end{tabular}
\caption{Main ion channels found in cervical cancer cells.}
\end{table}
mammals (Kv11.1, Kv11.2 and Kv11.3). To date, Kv11.1 expression has been shown in primary leukemias, colon cancer and endometrial tumors (22). The authors suggested h-erg channels as a potential tool discriminating endometrial cancer from simple hyperplasia (31). Kv11.1 also interacts with integrins to regulate survival and migration, and is involved in the regulation of apoptosis (28). Table 3 shows a summary of the main ion channels expressed in cervical cancer.

**Eag1 channels as early markers for cervical cancer**

Eag1 mRNA expression was found in 100% of cervical cancer human biopsies while only in 33% of normal control samples (pap smears negative). Interestingly, these controls were associated with various diseases such as HPV infection, endometrial hyperplasia, and ovarian tumors, suggesting that Eag1 could function as an early marker of cervical cancer (7). Therefore, researchers studied Eag1 expression in cervical pre-malignant lesions as well as Eag1 regulation by cervical cancer associated factors, namely, HPV infection and estrogens. Eag1 protein expression was found by immunochemistry in cervical cytologies from intraepithelial lesions, biopsies from cervical intraepithelial neoplasias (CIN 1, 2 and 3) and in normal smears from patients taking or not taking estrogens (36). Eag1 was found in 67% of the cervical cytologies from low-grade intra-epithelial lesions and in 92% of the samples from high-grade intraepithelial lesions, but only in 27% of the normal samples (36). Interestingly, morphologically normal cells obtained from dysplastic samples also exhibited Eag1 expression. In CIN biopsies, they found that the higher the grade of the lesion, the broader the Eag1 protein distribution (36). In addition, almost 50% of the normal patients taking estrogens displayed Eag1 expression suggesting Eag1 as a potential marker of cervical dysplasia and a risk indicator for developing cervical lesions in patients taking estrogens (36).

Noteworthy, Eag1 expression was absent in normal keratinocytes, but it was clearly upregulated in keratinocytes expressing E6, E7 or both HPV oncogenes. Interestingly, estradiol upregulated Eag1 expression in normal cultured trophoblasts and human vascular endothelial cells as well as in HeLa cervical cancer cells transfected with ERα. Thus Eag1 regulation by HPV oncogenes and estrogens, and its expression in pre-malignant lesions, strongly support the use of Eag1 as an early marker of cervical cancer (36).

Ion channel expression in cervical cancer cells offers new alternatives not only for early diagnosis but also for treatment and prognosis of the disease. With no doubt, the use of ion channels as markers and therapeutic targets of cervical cancer should help to reduce mortality from this disease.
Ion channels in cervical cancer

References


33. Pardo, L.A; Del Camino, D; Sánchez, A; Alves, F; Brüggemann, A; Beckh, S; Stühmer, W. 1999. Oncogenic potencial of EAG K+ channels. The EMBO Journal 20: 5540-5547.