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Review

DNA methylation of channel-related genes in cancers☆

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ABSTRACT

DNA methylation at CpG sites is an epigenetic mechanism that regulates cellular gene expression. In cancer cells, aberrant methylation is correlated with the abnormalities in expression of genes that are known to be involved in the particular characteristics of cancer cells such as proliferation, apoptosis, migration or invasion. During the past 30 years, accumulating data have definitely convinced the scientific community that ion channels are involved in cancerogenesis and cancer properties. As they are situated at the cell surface, they might be prime targets in the development of new therapeutic strategies besides their potential use as prognostic factors. Despite the progress in our understanding of the remodeling of ion channels in cancer cells, the molecular mechanisms underlying their over- or down-expression remained enigmatic. In this review, we aimed to summarize the available data on gene promoter methylation of ion channels and to investigate their clinical significance as novel biomarkers in cancer. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

Contents

1. Introduction

In humans, tumorigenesis is a process whose steps are the consequences of genetic alterations that will result in the transformation of normal cells into malignant derived cells. Progressive cell transformation implies changes in numerous cell signaling occurrences that ultimately lead to the acquisition by cancer cells of specific capabilities such as uncontrolled proliferation, resistance to apoptosis and up-regulated migration and invasion that are mainly acquired after changes

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in the cell genome [1]. Even though mutation of specific genes can explain cell transformation from normal to cancer cell, epigenetic mechanisms, such as changes in DNA methylation status can also be involved. Among all genes that have been reported to be targets of such regulatory mechanisms, genes encoding ion channels are potential targets. This latter point is especially important since an increased number of studies clearly show that the dysregulation of ion channel expression is involved in tumorigenesis and cancer cell properties such as proliferation, cell survival and invasiveness or resistance to therapies [2–5]. Ion channels have also been used as diagnostic and prognostic biomarkers of cancer [6,7] and have also been used for targeted therapy [8,9]. As their expression is up or down regulated in cancer and as actively transcribed genes are generally associated with promoter hypomethylation and inactive promoters with DNA hypermethylation, the DNA methylation status of some ion channel gene promoters has been investigated in cancer. This review summarizes our current knowledge regarding the implication of the methylation process of various ion channel genes in different cancers.

2. DNA methylation

Methylation consists of the addition of a methyl group on a nucleotidic base of the DNA through the action of the DNA methyltransferase enzymes (DNMTs) [10]. In Eucaryotes, only cytosine belonging to CG dinucleotides can be methylated, in the 5′ position, leading to the formation of 5-methylcytosine. These CG dinucleotides are also named CpG, in reference to the phosphodiester bond between the cytosine and the guanine. These CpG are distributed in islands, particularly concentrated in promoter regions of genes. CpG islands have a frequency of CpG dinucleotides approximately five times greater than the genome and represent 1–2% of the whole genome. In human, about 50–70% of genes have a promoter rich in CpG islands [11,12]. The methylation of CpG islands plays an important role in gene regulation, and usually represses their transcription.

Methylation patterns are established during the first cell divisions after fertilization, once the parental DNA has been demethylated. Once established, at each cell division, the methylation patterns are faithfully transmitted to daughter cells. However, methylation is proven to decline with age [13]. Some CpG islands undergo differential allelic methylation. This is the case of the CpG islands localized on the X chromosomes in females that are methylated on the inactive X chromosome. This is also the case of many imprinted genes.

2.1. DNA methylation in cancer

Carcinogenesis requires multiple genetic alterations that either drive cellular division (amplification of oncogenes) or remove checkpoints regulating this process in normal cells (inactivation of tumor suppressor genes), leading to the uncontrolled growth of cells, without consideration for the organism's need. In the past decades, many studies have shown that epigenetic changes, including DNA methylation modifications, also play a role in cancer development and progression [14]. From these observations, at least three major mechanisms by which CpG methylation can contribute to the oncogenesis can be distinguished. The first is the hypomethylation of the cancer genome that can lead to the activation of putative oncogenes, and enhances the genome instability. The loss of methylation can be localized to particular types of repetitive elements or to chromosomal domains [15]. The second is the focal hypermethylation of the promoters of tumor suppressor genes that inactivate their transcription. This mechanism is highly prevalent and well illustrated by the hypermethylation of the promoters of *RB1* in retinoblastoma [16], of *MLH1* in colon cancer [17,18], and of *BRCA1* in breast cancer [19]. The third is direct mutagenesis, by the fact that methylated CpG sites are hotspots for C to T transition mutations caused by spontaneous hydrolytic deamination. Additionally, methylation of CpG islands also promotes the binding of chemical carcinogens, and increases the rate of UV-induced mutations [20].

3. Hypermethylation of ion channel gene promoter and cancer (Table 1)

3.1. Chloride channels

The *CFTR* (cystic fibrosis transmembrane conductance regulator) is a member of the ATP-binding cassette transporter family whose promoter methylation is generally associated with gene inactivation whereas its expression is correlated with promoter unmethylation [21]. Indeed, the *CFTR* is one of the CpG-rich promoters where the CpG sites are not methylated in high and low *CFTR*-expressing cell lines. However, the CpG sites are partially or completely methylated in the very low or non-CFTR-expressing cell lines [21,22].

In cancer, the *CFTR* gene is reported to be frequently hypermethylated in bladder cancer [23], in hepatocellular carcinoma [24,25], and in breast cancer [26]. Recently Son et al. [27] has reported aberrant methylation associated with *CFTR* downregulation in 139 samples from patients with non-small cell lung cancer (NSCLC). Otherwise, the methylation of the *CFTR* promoter was related to a loss of gene expression and treatment of A549 lung cancer cells by the demethylating agent 5-aza-2′-deoxycytidine (5-Aza-dC) induces the re-expression of *CFTR* mRNA in these cells which support the role of methylation as a mechanism of *CFTR* gene inactivation.

In agreement with this ascertainment a correlation between the degree of CpG methylation and the level of *CLCA2* (calcium-activated chloride channel) gene expression was reported in breast cancer tissues and cell lines [28]. By using bisulfite DNA sequencing, they demonstrate that the promoter region is hypermethylated in tumor cells that show no or low *CLCA2* expression, while, *CLCA2*-promoter methylation is not detected in samples, derived from normal breast tissue, as well as in normal epithelial breast cells (MCF-10A). Moreover, methylation of the *CLCA2* promoter region was reduced in cell lines treated with 5-Aza-dC agent, while the expression of CLCA2 RNA was restored. This property, as well as the ensuing consequences, leads Li et al., [28] to suggest *CLCA2* as a tumor suppressor gene in breast cancer. In full agreement with this consideration, it was shown that transfecting breast cancer cell lines (MDA-MB-435 and MDA-MB-231) with *CLCA2* under the control of a promoter resistant to inhibitory hypermethylation reduces migration and invasion, but failed to change growth rate or anchorage-independent growth of these cells in soft and hard agar [29]. Alongside, nude mice injected with MDA-MB-231 cells transfected with *CLCA2* produce few tumors and exhibit either reduced tumor size or no lung tumors when compared with MDA-MB-231 control inoculated mice [29]. Moreover, the high-level expression of m*CLCA2* correlated closely with the onset of apoptosis [30]. Altogether, these data suggest that loss of *CLCA2* expression in human breast cancer is likely associated with tumorigenicity [29].

3.2. Calcium channels

Basically, calcium channels are implicated in many biological processes potentially relevant to the malignant process including proliferation, apoptosis and metastasis [31,32]. Both *CACNA1G* (T-type Ca^{2+} channel) and *CACNA2D3* (calcium channel regulatory subunit α2δ-3) have numerous properties consistent with tumor and/or metastasis suppressor function as demonstrated by their ectopic expression that inhibits breast and gastric cell growth, whereas the knockdown using inhibitory RNA or pharmacological tools leads to increased gastric and breast cancer cell proliferation [33,34], and breast cancer cell apoptosis [34]. Further evidences supporting the role of *CACNA2D3* as a tumor suppressor gene in lung cancer, renal cancer, neuroblastoma and osteosarcoma have been reported [35–39].

Hypermethylation of *CACNA1G* is detected in various human primary tumors: colorectal cancers (≈35%), gastric cancers (≈25%), acute myelogenous leukemia cases (≈13%) [40], and pancreatic adenocarcinoma (≈16%) [41]. It is proposed that inactivation of *CACNA1G* may play a

Table 1
Aberrant methylation of ion channel gene promoter in cancer.

Gene symbol	Gene name	Tumor type	Methylation status	Correlation with clinical parameters	References
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator (ATP-Binding Cassette Sub-Family C, Member 7)	Lung cancer Non-small cell lung cancer (tissue and cell lines)	Hyper methylation	• More frequently observed in squamous carcinomas than adenocarcinomas • Associated with poor survival among younger patients	[27]
		Bladder cancer	Hyper methylation	• Marker of bladder cancer	[23]
		Hepatocellular carcinoma (tissue samples)	Hyper methylation	n.d.	[24]
		Hepatocellular carcinoma (tissue samples)	Hyper methylation	• Potential marker of well-differentiated hepatocellular carcinoma	[25]
		Breast cancer MCF-7 cell line	Hypermethylation	n.d.	[26]
CLCA2	Chloride Channel Accessory 2	Breast cancer	Hyper methylation	n.d.	[28]
CACNA1G	Calcium channel, voltage-dependent, T type, alpha 1G subunit ($Ca_v3.1$)	Colorectal cancer	Hypermethylation	• Correlation with methylation of p16	[40]
		Colorectal adenoma	Hypermethylation		
		Gastric cancer	Hypermethylation		
		Acute myelogenous leukemia	Hypermethylation		
		Pancreatic adenocarcinoma	Hypermethylation	n.d.	[41]
CACNB2	Calcium channel, voltage-dependent, Beta 2 subunit	Esophageal adenocarcinoma	Hypermethylation		[53]
CACNA2D3	Calcium channel, voltage-dependent, Alpha 2/Delta Subunit 3	Gastric cancer diffuse type	Hypermethylation	• Correlation with poorer outcome	[33]
		Intestinal type	Hypermethylation		
		Breast cancer	Hypermethylation	• Frequently methylated in breast cancer that metastasized to the CNS • Association with increased risk of recurrence, particularly at visceral sites of liver and lung	[42]
		Gastric cancer	Hypermethylation	• Correlation with physical activity	[56]
KCNA3	Voltage-gated potassium channel, shaker related subfamily, member 3 ($K_v1.3$)	Breast cancer	Hypermethylation	• Poor differentiation (SBR histological grade III)	[45]
		Pancreatic cancer	Hypermethylation	• Associated with poor patient survival	[44]
		Lung, ovarian, kidney, prostate colon cancer	Hypermethylation		[46]
KCNH2	Potassium voltage-gated channel, Subfamily H (Eag-related), Member 2 (HERG, $K_V11.1$)	Ovarian cancer	Hypermethylation	• A good prognostic marker in ovarian cancer	[48]
		Melanoma	Hypermethylation	n.d.	[47]
KCNH5	Potassium voltage-gated channel, Subfamily H (Eag-related), Member 2 (Kv10.2, EAG2)	Lung cancer	Hypermethylation	n.d.	[52]
		Esophageal adenocarcinoma	Hypermethylation	n.d.	[53]
		Non-small cell lung cancer	Hypermethylation	• More frequent in adenocarcinomas compared to squamous cell carcinomas	[54]
KCNH5 & KCNH8	Potassium voltage-gated channel, Subfamily H (Eag-related), Member 5 (Kv10.2, EAG2) & 8 (ELK, ELK1, elk3, Kv12.1)	Non-small cell lung cancer	Hypermethylation	• More frequent in females compared to males	[54]
KCNN2, *KCNIP1*, *KCNQ5*, *KCNK9*, *KCNE2*, *KCNG1*, *KCNA6*, *KCNV1*, *KCNAB3*, *KCNJ15*, *KCNJ8*	K^+ channels (KCN family)	Esophageal adenocarcinoma	Hypermethylation	n.d.	[53]
CACNA1H	α1H T type Ca^{2+} channel (Cav3.2)	Adult T-cell leukemia	Hypomethylation	n.d.	[74,75]
KCNH5	Potassium voltage-gated channel, Subfamily H (Eag-related), Member 2 (Kv10.2, EAG2)	Melanoma	Hypomethylation		[78]
KCNN4	Potassium intermediate conductance calcium-activated channel, subfamily N, member 4 (KCa3.1, SK4…)	Lung cancer	Hypomethylation	• Strong indicator of poor prognosis in lung cancer	[98]
KCNQ1OT1	KCNQ1 opposite strand/antisense transcript 1	Adrenocortical adenomas and carcinomas	Hypomethylation	n.d.	[99]
Aquaporin 1	Aquaporin 1 (water channel protein)	Salivary gland adenoid cystic carcinoma	Hypomethylation	n.d.	[77]

role in cancer development by means of modulating calcium signaling, that potentially affects, at a time, cell proliferation and apoptosis [40].

Hypermethylation of *CACNA2D3* is concomitant with its down-regulated expression in breast cancer cell lines, primary cancers and metastatic lesion tumors [42], as well as in gastric cell lines and tissue samples [33]. In contrast, the CpG islands are unmethylated in normal gastric and breast tissues as well as in corresponding normal cell lines. In this case as well, 5-Aza-dC upregulates the expression of *CACNA2D3* in both breast and gastric cancer cells that exhibit CpG island methylation but fails to affect cells lacking methylation. Moreover, in vitro exogenous *CACNA2D3* expression inhibits cell growth and adhesion and up regulates p21 and p27 expression in gastric cancer cell lines with inverse effects with *CACNA2D3* small interfering RNA treatment [33]. These results are consistent with methylation-dependent transcriptional silencing being the mechanistic basis of *CACNA2D3* downregulation in breast and gastric cancer.

3.3. K^+ channels

Potassium channels have been reported to play various roles in cancer progression as well as cell proliferation, apoptosis, migration, and invasion during metastasis [2,4,5,43].

A methylation of *KCNA3* (Kv1.3 potassium channel) gene promoter and its association with the decrease of the expression of Kv1.3 channel were first published in breast and pancreas adenocarcinomas [44,45]. More recently, *KCNA3* promoter hypermethylation was also identified as one of the most prevalent events in tumorigenesis affecting various tumor types including lung, ovarian, kidney, prostate and colon [46]. Recently, an inverse relationship between methylation and expression of *KCNH2* (hERG1, Kv11.1) was also found to be associated with clear-cell ovarian cancer (171 tissue samples) and lymphoma, highlighting the well-established role of CpG island hypermethylation on repression of gene expression [47,48]. Given that overexpression of hERG1 promotes proliferation, migration and metastasis in ovarian cancer [49,50], it was suggested that loss of expression of hERG1 by methylation could be a good prognostic marker in ovarian cancer [48].

The *KCNH5* gene (also known as EAG2 or Kv10.2), encodes an EAG voltage-gated potassium channel that is involved in the regulation of cell cycle and proliferation [51]. The methylation of *KCNH5* gene was also identified in lung cancer [52]. *KCNH5* is respectively methylated and hypermethylated in 80% and 53% of NSCLC tissues [52]. Interestingly, when *KCNH5* gene promoter is methylated in cancer tissue, a slight methylation (14%) is found in the adjacent noncancerous lung tissues. On the other hand, *KCNH5* is hypermethylated in cancerous tissues and no methylation is observed in noncancerous tissues [52]. Furthermore, hypermethylated CpG sites of KCN genes, that encode for a large number of K^+ channel families (Table 1), have also been reported in esophageal carcinoma [53].

3.4. Link between ion channel promoter aberrant methylation and clinical outcomes in cancer (Table 1)

To our knowledge, there are few studies that address a possible link between ion channel promoter aberrant methylation and different clinical outcomes in cancer (Table 1).

A study performed on a cohort of 139 non-small cell lung cancer (NSCLC) samples, among which were 60 squamous cell carcinoma (SCC) and 79 adenocarcinoma (AC), and the matched non-malignant lung tissue samples shows methylation of CpG islands in the *CFTR* gene promoter in 30.2% (vs. 18.7%) of the studied tissue samples. The *CFTR* methylation is associated with loss of *CFTR* gene expression and its frequency is significantly higher in tissue samples from SCC than AC [27].

Regarding the survival of patients with NSCLC, there is no correlation between promoter methylation of the *CFTR* gene and the patient's prognosis. However, *CFTR* methylation is associated with significantly poorer survival among younger patients vs. elderly patients. The prognostic and predictive factors identified in this study may be useful in considerations of which patients are likely to benefit from adjuvant therapy [27].

Another study, conducted on samples from patients with bladder cancer, highlights a strong methylation profile of an 11-gene set including *CFTR* (which ranks second) in urine sediments. This makes these genes and the *CFTR* in particular a sensitive and specific tool for detection of bladder cancer [23].

The methylation of *KCNA3* (Kv1.3 K^+ channel) is correlated to SBR histological grade III in breast cancer [45]. Indeed, the methylation of the *KCNA3* promoter is associated with poor differentiation inasmuch as 54% of methylation is found in grade III tumors versus only 13% in grade I tumors. In grade II tumors, Kv1.3 gene promoter methylation is observed in 52% of the cases. Otherwise, no difference was observed between methylated and unmethylated tumors in terms of lymph node status, estrogen and progesterone receptor expression, ErbB2 expression and expression of the Ki67 proliferation index [45]. The same authors have pointed out an association between Kv1.3 hypermethylation and the survival rate in patients with pancreatic cancer, although the association did not reach statistical significance [44].

Methylation patterns of *KCNH5* (Kv10.2, EAG2) and *KCNH8* (ELK, ELK1, elk3, Kv12.1) varied depending on histological type of lung cancer [54]. Both gene promoters are hypermethylated in non-bronchoalveolar adenocarcinomas compared to squamous cell carcinomas. Furthermore, both *KCNH8 and KCNH5* hypermethylation was more frequent in females compared to males [54]. However, this study, performed on a small sample, deserves to be checked with a greater number of samples.

In a study on gastric cancer, *CACNA2D3* (calcium channel, voltage-dependent, alpha 2/delta subunit 3) is proposed to have tumor suppressive functions [33]. Indeed, its overexpression in *CACNA2D3*-positive HEK-293T and *CACNA2D3*-negative NUGC4 cell lines significantly inhibits cell growth of transfected cells versus controls. It also inhibits cell adhesion and upregulates p21 and p27 expression in both transfected cell lines. In this regard, it is reported that patients with gastric cancer which do not express p27 protein have worse clinical outcomes than patients with intact p27 expression [55] suggesting that *CACNA2D3*-negative gastric cancers are suspected to be more aggressive than positive ones with respect to p27 expression rate. Otherwise, survival of patients with *CACNA2D3*-methylated gastric cancers is significantly shorter than that of patients whose *CACNA2D3* is non-methylated. These findings support the proposal that methylation of *CACNA2D3* is a useful prognostic marker for patients having advanced gastric cancer.

Besides, *CACNA2D3* methylation status is conversely correlated to physical activity (i.e., more frequently found in gastric carcinoma patients having no physical activity than in those with physical activity). As a consequence of this, Yuasa et al. [56] hypothesize that some lifestyle factors may influence gastric cancer development.

Recently, *CACNA2D3* CpG island methylation has been investigated in central nervous system metastases derived from primary breast carcinomas [42]. The results show that *CACNA2D3* transcripts are regulated, in breast cancer cell lines, through the DNA methylation in the promoter-associated CpG islands. This methylation is upregulated by pharmacological demethylation using Azacytidine. Moreover, this promoter-methylation is regularly observed in breast cancer that metastasizes to the central nervous system. Furthermore, in a large series of 100 primary breast carcinoma samples, an association is pointed out between methylation and increased risk of cancer recurrence, particularly at visceral sites of liver and lung. This may contribute to breast cancer metastatic phenotype [42].

3.5. Other channels and transporters

Many studies have shown that other channels and transporters encoding genes could be the target of methylation or demethylation processes and involved in cancer or cell transformation. Thus, a study from Xu and collaborators described, in a genome-wide methylation analysis, the methylation of several genes in esophageal adenocarcinoma [53]. Among all of these genes, they reported genes that have been described to be methylated for a long time but also new and unusually frequent hypermethylated genes such as membrane transporter and ion channel genes. Thus, they reported the hypermethylation of *SLC18A3* and *SLC6A2*, a vesicular acetylcholine (Ach) transporter and a norepinephrine transporter, respectively. More interestingly, they reported the hypermethylation of *CACNB2* (gene encoding for the beta 2 subtype of voltage-dependent Ca^{2+} channel), and *CHRNA3* (gene encoding for the neuronal Ach receptor α3 subunit).

Xia et al. [57] reported that *ASIC2* (the gene that encodes the Acid Sensing Ion Channel 2) is also a potential target for methylation. Indeed, they showed that its differential regulation of expression occurs between high-grade glial-derived tumor cells and normal astrocytes. In one study, they showed that *ASIC2* mRNA is absent in the majority of glioma cells [58] whereas both *ASIC1* and *ASIC2* mRNAs are present in both normal brain tissue or from low-grade or benign tumors. By the use of methylation-specific PCR experiments, they revealed that four glioma cell lines which do not express *ASIC2* or express a very low level of *ASIC2* have DNA methylation in the CpG island of the promoter. In the same way, they showed that in 12 freshly resected

human glioblastoma multiform tissues, 7 samples have promoter methylation and that *ASIC2* gene expression is mostly absent or at low levels.

It has been shown that *ENSA* (endosulfine alpha), which is a ligand of sulfonylurea receptor that is coupled to KATP channels involved in insulin release process, is a bivalent gene in mesenchymal stem cells that is involved in cell differentiation and transformation [59,60]. Interestingly, in breast cancer cell lines, *ENSA* methylation state changes in response to environmental signals such as changes in estrogen [61]. In a recent work, Chen et al. [62] showed a deregulated *ENSA* methylation in liver and breast cancers. Their methylation state was opposite to each other, indicating lineage-specific methylation changes. Indeed, *ENSA* promoter was hypomethylated in liver cancer but was hypermethylated in breast cancer. They determined that *ENSA* expression correlates with attenuated tumor propagation in liver cancer and that *ENSA* hypomethylation in liver cancer usually indicates gain of *ENSA* function in tumorigenesis. They showed also that the DNA methylation inhibitor (5-Aza-dC) was able to induce an elevation of *ENSA* expression and that *ENSA* suppressed the hepatic tumor growth in cultured cells and in mice xenograft models through an interaction with microtubule-associated serine/threonine-protein kinase-like. They speculated that silenced *ENSA* function might be essential for liver cancer initiating cells but not necessary for tumor expansion in liver cancer.

3.6. Ionotropic receptors in cancer (Table 2)

The ionotropic glutamate receptors (N-methyl-D-aspartate receptors (NMDARs)) are oligomeric protein complexes that form ion channels in the plasma membrane that enable ion fluxes (e.g., Ca^{2+}) known to be crucial for cellular properties such as synaptic transmission, muscular cell contraction or cancer cell migration and survival [63,64]. Recently, several studies highlight their role in cancer cell growth and propose them as potential therapeutic targets [65–67].

High methylation of *GRIN2B* and *GRIN2A* (genes that encode NMDAR2B and -2A respectively) are reported to be tumor suppressors in different carcinomas. *GRIN2B* is highly methylated in esophageal, head and neck squamous carcinomas, and gastric cancer [68,69]. The methylation of *GRIN2B* is associated with the silencing of NMDAR2B expression. NMDAR2B expression is reactivated by a treatment by a well-known DNA methyltransferase inhibitor (5-Aza-dC). Moreover, ectopic expression of NMDAR2B in cells that do not express NMDAR2B leads to drastic cell apoptosis in esophageal cancer and to a decrease of colony gastric cancer forming ability. *GRIN2B* is either proposed as a high cancer frequency biomarker [68] or may serve as an important molecular marker in serum/plasma DNA for early cancer detection and monitoring [69]. *GRIN2B* has also recently been found to be highly methylated in lung cancer cell lines and cancer tissues from adenocarcinomas and large cell carcinomas where it is upregulated following the use of 5-Aza-dC [70]. Interestingly, *GRIN2B* methylation is associated with a better prognosis in squamous cell carcinoma [70].

On the other hand, *GRIN2A* (the gene that encodes NMDA2A) was studied in colorectal cancer (CRC) cell lines and Human CRC tissues versus corresponding normal adjacent mucosa [71]. *GRIN2A* is hypermethylated in 100% of the studied cancer cell lines and is reactivated using 5-Aza-dC. Moreover, *NMDR2A* shows tumor suppressive activity as assessed in transfected-HTC116 cells which is found to regulate the early stage of apoptosis. These results have led Kim et al. [71] to propose *GRIN2A* as a potential diagnostic and therapeutic target molecular marker for colorectal cancer.

Finally, *GRIK2*, which encodes Glutamate Receptor, Ionotropic, Kainate 2 (GLUR6), has also been reported as a tumor-suppressor in gastric cancer [72]. *GRIK2* hypermethylation in gastric cancer is associated with loss of its gene expression. Moreover, *GRIK2*-expressing gastric cancer cell lines are both unable to migrate and fail to form colonies. These results allow for the proposal of *GRIK2* as a potential tumor marker for gastric cancer.

4. Hypomethylation of ion channel gene promoter and cancer (Table 1)

Because cancer-linked DNA hypomethylation might occur early in oncogenesis, very few studies reported a correlation between hypomethylated channels and tumoral processes.

The first related one was *CACNA1H* gene (α1H T type Ca^{2+} channel) is normally transcribed in kidney and heart [73]. Authors showed that in *CACNA1H* gene, CpG sites in both the 5′ and 3′ regions were hypomethylated in Adult T-cell Leukemia, and *CACNA1H* gene was aberrantly transcribed, suggesting a link between DNA hypomethylation and the aberrant transcription [74]. It has been reported that antagonists of T-type Ca^{2+} channels inhibited cell proliferation [75] suggesting that its aberrant expression is associated with cell proliferation [74].

Aquaporins (AQPs) are water channels known to be formed by identical subunit proteins. Among isoforms of aquaporins, *AQP1* is expressed in different human cancers where it is involved both in cell proliferation and migration [76].

In primary Adenoid Cystic Carcinoma (ACC) tissues, *AQP1* is both hypomethylated and overexpressed at mRNA and protein levels when compared to normal salivary tissue [77]. Interestingly, treatment by demethylating agents of ACC-derived SACC83 cell line, in which *AQP1* is methylated and exhibits low expression, induces *AQP1* re-expression agreeing for an epigenetical regulation of *AQP1* expression. Moreover, transient expression of *AQP1* leads these cells to proliferate and form large size colonies versus control cells. Furthermore, silencing *AQP1*, in non-small cell lung carcinoma cell lines which show high *AQP1* expression, leads to the inhibition of cell growth. On the basis that silencing of *AQP1* inhibited cancer cell growth and that the expression of *AQP1* was regulated by promoter demethylation, *AQP1* is proposed as a potential novel therapeutic target and a promising oncogene candidate in Adenoid Cystic Carcinoma [77].

Table 2
Hypermethylation of glutamate receptor, ionotropic, NMDAR (2A and 2B) and Kainate 2.

Gene symbol	Gene name	Tumor type	Correlation with clinical parameters	References
GRIN2B	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B (NMDAR2B)	Lung (adenocarcinoma large cell carcinoma) (adeno-squamous cell carcinoma) cell lines Squamous carcinoma	No correlation among gender, age, smoking, histological type, or stage Better survival Better prognosis	[70]
		Esophageal and head and neck squamous cell carcinomas(ESCC) cell lines,	n.d.	[68]
		Esophageal squamous cell carcinoma gastric cancer cell lines	n.d.	[69]
GRIN2A	Glutamate receptor, ionotropic, N-methyl D-aspartate 2A (NMDAR2A)	Colorectal cancer cell lines	n.d.	[71]
GRIK2	Glutamate receptor, inotropic, kainate 2 (GLUR6)	Gastric cancer cell lines	n.d.	[72]

Recently, Macaulay et al. [78] established that hypomethylation of the human placental-specific *KCNH5* promoter, a retrotransposon-derived transcript of the EAG voltage-gated potassium channel gene, is frequently associated with *KCNH5* expression in melanoma cells. Since EAG and *KCNH5* have been involved in cell cycle regulation, proliferation and tumor progression in cancer [79,80], the methylation state of this gene appears as an interesting hallmark in melanoma [78].

Moreover, at the last meeting of the German Physiological Society, Schwab's team demonstrated that KCa3.1 channel gene (*KCNN4*) promoter is hypomethylated in an aggressive non-small cell lung carcinoma cell line and in patient samples [81]. The loss of DNA methylation of the *KCNN4* promoter was associated with an increased KCa3.1 channel expression and function, both findings being strong indicators of poor prognosis in lung cancer.

5. Potential clinical aspects and pharmacological tools

Aberrant promoter methylation has been described for several genes in various malignancies and the wide spectrum of genes involved suggest that specific tumors may have their own distinct methylation profile [82,83].

Studies on aberrant methylation of channel-related genes are increasingly emerging. The available data from recent studies in several types of cancer show that aberrant methylation of ion channels is a cancer-specific finding, since it is significantly observed in cancer and is not likely to be found in healthy control tissue. Moreover, a growing body of evidence has confirmed the correlation of *CFTR*, *KCNA3*, *CACNA2D3* hypermethylation and *KCNN4* hypomethylation with several clinicopathological parameters, including advanced disease (poor differentiation), recurrence, and disease-specific survival. However, more prospective studies are needed to affirm the clinical use of ion channel methylation status (hypermethylation, hypomethylation) as a fully independent prognostic marker in larger groups of patients.

As presented in this review, destabilization of DNA methylation patterns (hypermethylation or hypomethylation) increases tumorigenesis. Such epigenetic events have been used for treatment, by increasing or decreasing methylation processes [84].

For example, a therapeutic strategy blocking DNA methylation with 5-azacytidine [85] and 5-aza-2′-deoxycitidine has been approved by the Food and Drug Administration for treatment of preleukemic myelodysplastic syndrome [86]. In the 1980s, these compounds were found to have hypomethylating activity after incorporation into the DNA of actively replicating tumor cells [87,88]. They have since been used in clinical trials for several forms of cancer [89]. In vitro investigations have demonstrated that these compounds may reverse abnormal DNA methylation and eventually restore normal gene expression profiles in various cancer cell lines [90]. Nevertheless, it has been suggested that the effects of chemotherapeutic agents inducing hypomethylation may be beneficial in the short-term, but they may allow for progression and recurrence from cancer cells that survive or are even enhanced by DNA hypomethylation [91].

On the other hand, pharmacological tools inducing DNA methylation may be a beneficial approach like for Glioblastoma Multiform patients [92]. Indeed, temozolomide (TMZ) an oral alkylating agent has been shown to exhibit great antitumor activity, by activating mismatched repair mechanisms and DNA damage signaling pathways, leading to G2/M cell cycle arrest and eventually to induction of cell death [92–94]. However, its use in the treatment of Glioblastoma Multiform was limited due to its insufficient delivery across the blood–brain barrier and its resistance to the drug. Interestingly, Ningaraj et al. [95] showed that intravenous administration of ATP-sensitive potassium channel activator (minoxidil sulfate) increases TMZ delivery to brain tumors. At the same time, they also demonstrated that co-infusion of a calcium-dependent potassium (KCa3.1, SK4) channel agonist (NS-1619) with TMZ and herceptin resulted in enhanced drug delivery to brain-tumor cells [96]. In this context, KCa channel agonists may benefit brain tumor patients by increasing an anti-neoplastic agent's delivery to brain tumors. This original combination has led to various studies that use a mixed therapy approach where TMZ is combined with either radiation therapy or other anti-cancer agents [97].

Although methylation of ion channels is still a novel area of research in oncology, the development of the field might enable the identification of subgroups of patients with poor prognosis, who might require a different therapeutic approach.

6. Conclusions

It is now widely recognized that epigenetic alterations are associated with the process of neoplastic transformation. Epigenetic alterations of ionic channel-related genes may contribute to carcinogenesis by

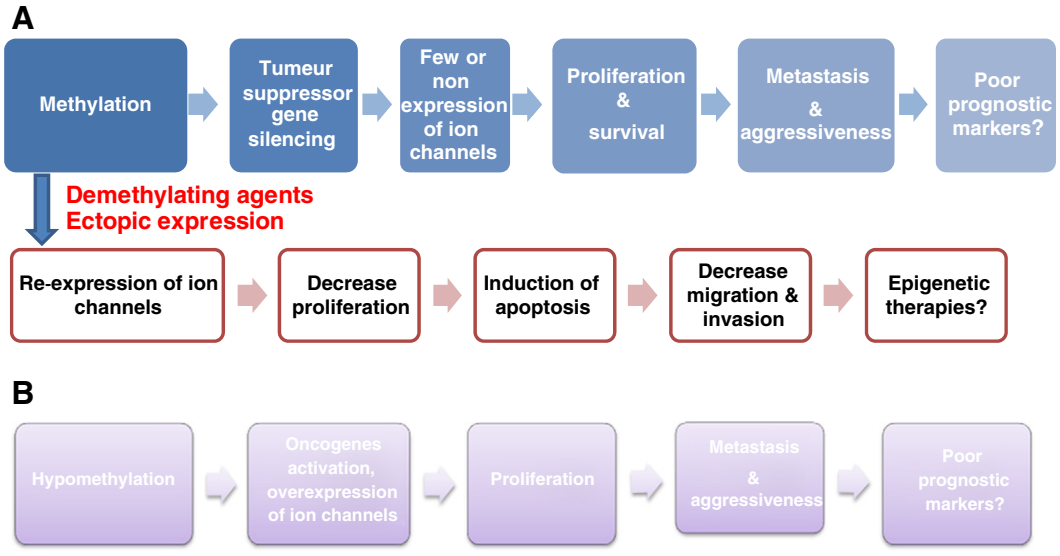

Fig. 1. Aberrant epigenetic modifications of ion channels in cancer. A) During tumorigenesis, silencing gene expression of ion channels is associated with their promoter hypermethylation. Methylation-mediated silencing is reversed either upon treatment with DNA methyltransferase inhibitors, or experimental re-expression of ion channels leading to decrease of proliferation and metastasis aggressiveness and increase of apoptosis. B) Hypomethylation of ion channel gene promoter mediates oncogene activation leading to the development and progression of cancer.

regulating their expression (Fig. 1). Hypermethylation of ion channel gene promoter is frequent in breast, lung, and gastric cancer and is associated with loss of their expression. The restoration of their expression by an ectopic expression, or a treatment by demethylating agents, which inhibits cell proliferation, metastasis and increases apoptosis argues for their use as new potential therapeutic strategies for cancer (Fig. 1A).

Hypomethylation of ion channel gene promoters is less frequent than hypermethylation in cancer. Loss of DNA methylation in the promoter region of certain ion channel genes (*KCNN4*, *AQP1* and *KCNH5*) has been reported in non-small cell lung cancer, Adenoid Cystic Carcinoma, and melanoma that is associated with an increase of their expression. Furthermore, ion channel gene promoter hypermethylation as well as hypomethylation are strongly correlated with a poor prognosis, suggesting that both could potentially serve as a clinically useful prognostic factor (Fig. 1).

Conflict of interest statement

We declare no conflict of interest.

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