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Chloride Channels As Novel Biomarkers And Therapeutic Targets Against Cancer

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Abstract: The role of ion channels in the development of different cancer has become a novel area of research. There are so many ion channels which are responsible for cancer like phenotype. Among all chloride ion channel is considered to be one of the most active channels along with potassium in case of tumor genesis. High rates of proliferation, active migration, and invasiveness are all hallmarks of cancer which are caused due to channelopathy. It is known that all this action requires action of chloride channel activity as well as the action of ANOCTAMIN genes, which controls the expression of chloride channels in cancer. As the chloride channels involvement is very essential for cancer cell, and as it is known that the abnormal activity of chloride channels can promote the cancer so they can be used as possible aims to cure cancer. Here in this reviewthe chloride channels how they can acts as biomarkers of cancer and specifically which channels are responsible for cancer and ways of linking it for developing anti-tumor drugs will be discussed.

Keywords: **CLIC, CLCA, Oncochannelopathy, Tumorigenesis, Anoctamins.**

1. INTRODUCTION

Chloride channels are family of channels for the transfer of chloride ions[4,5].These channels can conduct other ions but its chloride concentration in-vivo is higher than other anions like SCN-,I- and HCO3-[4]. Each channel is composed of two similar subunit- each subunit containing one pore[5] .Each subunit is made up of two associated halves oriented in opposite directions to the anion opening [1,5]. The chloride channels family contains 10 or 12 transmembrane helices, each protein forms a single pore [3,5].

There are three binding sites present on the pores namely S int, S ext, S cen which bind to chloride and other anions [5]. S int is exposed to intracellular fluid similarly S cen at the centre and S ext at the extracellular portion [5]. Each binding site binds chloride anions simultaneously. CLC transporter moves H+ across the membranes [4,5].Two glutamate residues namely- GLUin and GLUex controls chloride movement between protein and extracellular fluid [5]. The gating in and out of ions occurs through two mechanisms- fast gating and slow gating. Slow gating involves both protein subunits closing their pores at the same time, while fast gating involves independent opening and closing of pores [5]. When the slow gate is closed, no ions can permeate through the pores [5]. Recent studies show a potential link between the chloride channels and the cancer [4]. Cancer cells resistant to cell death are associated with regulatory volume decrease (RVD; the renewal of cell capacity in response to hypoosmotic stress) that takes place due to higher expression of volume-regulated anion channels (VRACs) [4,10]. It is also foundto be linked with metastases process. Additionally, the chlorotoxin [neurotoxin isolated from the venom of

the giant yellow Israeli scorpion (Leiurusquinquestriatus) inhibitor to cl channels] is currently in phase I/II clinical trials for the treatment of malignant gliomas [13]. However as all the data is related with the development of cancer, to use the channels for diagnosis and treatment of cancer may be helpful in future. For this reason in this mini review we are trying to gather some current knowledge about chloride channels, and then discuss about its potency to acts as novel biomarker's and targets for cancer.

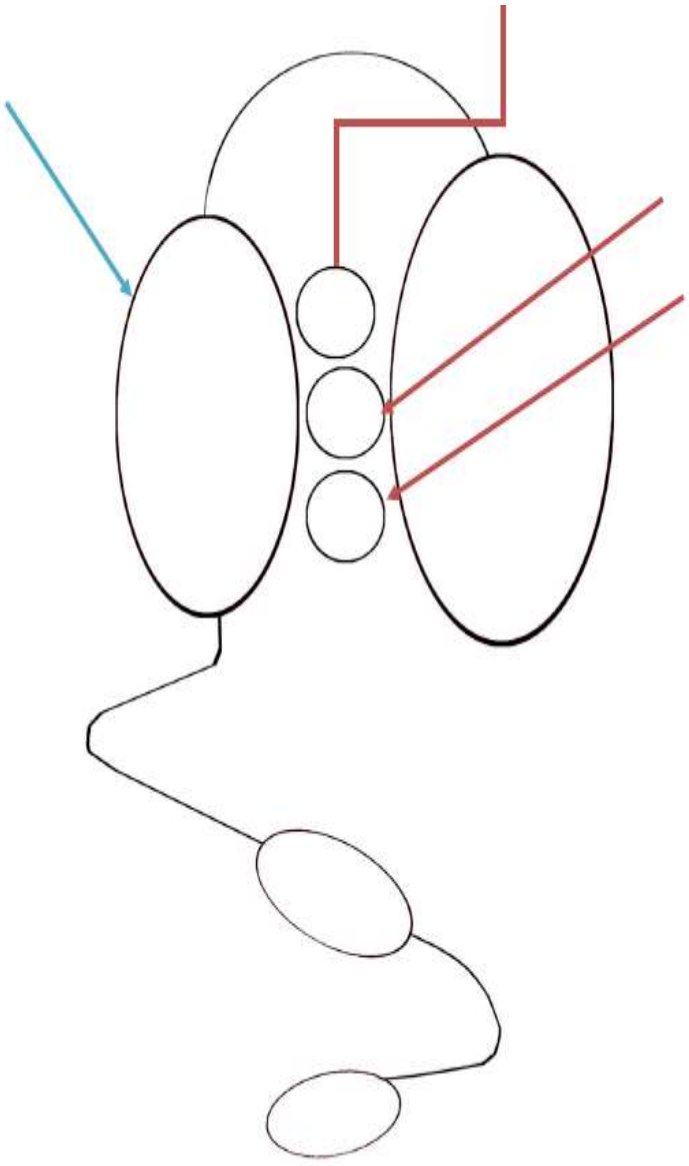

Fig.1 Structure of a typical chloride channel].Blue line- Transmembrane domain Brown lines- Anion binding sites {External, middle and internal}

2. TYPES OF CHLORIDE CHANNELS

Chloride channels family are normally classified into five major subfamilies namely-

1. Epithelial chloride channel family (E-CLIC)
2. Cystic fibrosis transmembrane conductance regulator (CFTR)
3. Chloride intracellular ion channel family (CLIC)
4. Calcium sensitive chloride channel
5. Voltage dependent chloride channel

Now, we will discuss about each ion channels in details.

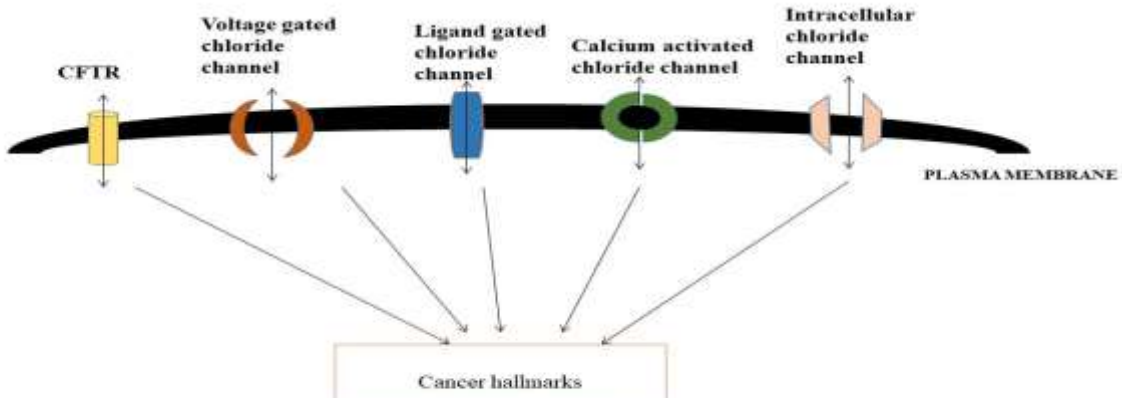

Fig. 2. All Chloride channels

2.1 E-CLIC

Members of this family catalyze bidirectional transport of chloride ions (Evans *et al*.,2004). Mammals have multiple isoforms of this epithelial protein (The Journals of biological chemistry by Evans SR). The first member of this family to be characterized was a respiratory epithelium; Ca2+ regulated chloride channel protein, isolated from tracheal membranes. It is characterized as a 140 kDa complex (Agnel M *et al*.,1999).

2.2 CFTR

It is a chloride channel belonging to the family of ABC transporters (ATP-binding cassette). The ATPase subunits utilize the energy of ATP binding and its hydrolysis to provide the energy needed for the translocation of substrates. The role of CFTR has been discussed in later parts [5,91].

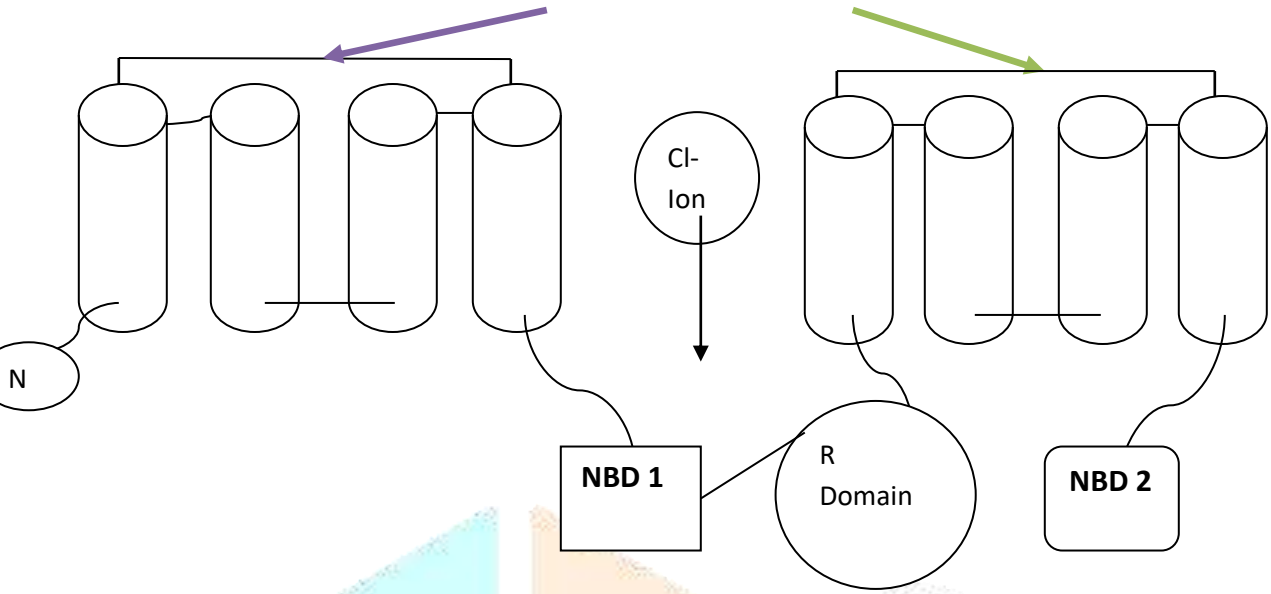

Fig.3 CFTR Chloride Channel, Purple arrow- MSD 1 Green arrow- MSD 2

2.3 CLIC Family

consists of six proteins in humans namely CLIC1, CLIC2 up to CLIC6. Members exist as both monomeric proteins and integral membrane proteins 5]. They possess one or two transmembrane alpha helical segments. CLIC proteins namely CLIC 1, 2 and 4 exhibit glutaredoxin like glutathione ependent oxidoreductase enzymatic activity (AI Khamici et al).

1. CLIC1- is a protein encoded in humans by CLIC1 gene and is a member of p64 family. It maintains cell volume, pH and membrane potential. It is detected in cell nucleus and plasma membrane. Oxidative stress, characterized by overproduction of reactive oxygen species (ROS), is a major feature of several pathological states. Indeed, many cancers and neurodegenerative diseases are accompanied by altered redox balance, which results from dysregulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [1,2].Following microglial activation, CLIC1 translocates from the cytosol to the plasma membrane where it promotes a chloride conductance. The resultant anionic current balances the excess charge extruded by the active NADPH oxidase, supporting the generation of superoxide by the enzyme [1,2].
2. CLIC2- is a protein encoded in humans by CLIC2 gene and is a member of p64 family. It maintains cell volume, pH and membrane potential. It is detected in liver and skeletal muscle tissue [2].
3. CLIC3- is a protein encoded in humans by CLIC3 gene [2,5].
4. CLIC4- is a protein encoded in humans by CLIC4 gene and is a member of p64 family. The CLIC4 gene is expressed in pancreatic cancer cell line [1,2]
5. CLIC5
6. CLCI6- is a protein encoded in humans by CLIC6 gene. This gene is found in chromosomes 1, 6 and 21 (Friedli M *et al.*, 2003).

2.4 Calcium sensitive chloride channel

he calcium sensitive chloride channels consist of four chloride channel accessory protein namely CLCA1 up to CLCA4, which are encoded by the orresponding CLCA genes respectively. Like CLCA1 is encoded by CLCA1 gene and so on [5]. The role of these ion channels in cancer has been iscussed in later part of the review.

2.5 Voltage dependent chloride channel

he voltage dependent chloride channels (VDC) consist of nine genes. These genes are CLCN1, CLCN2, CNCL3, CNCL4, CNCL5, CNCL6, LCN7, Ka and Kb. These are encoded by their corresponding genes that is CLCN1 by CLCN1 gene and CLCN 2 by CNCL2 and so on [5,16]. he exact role of CNCL in the cancer is not fully understood yet.

Chloride channels in cancer

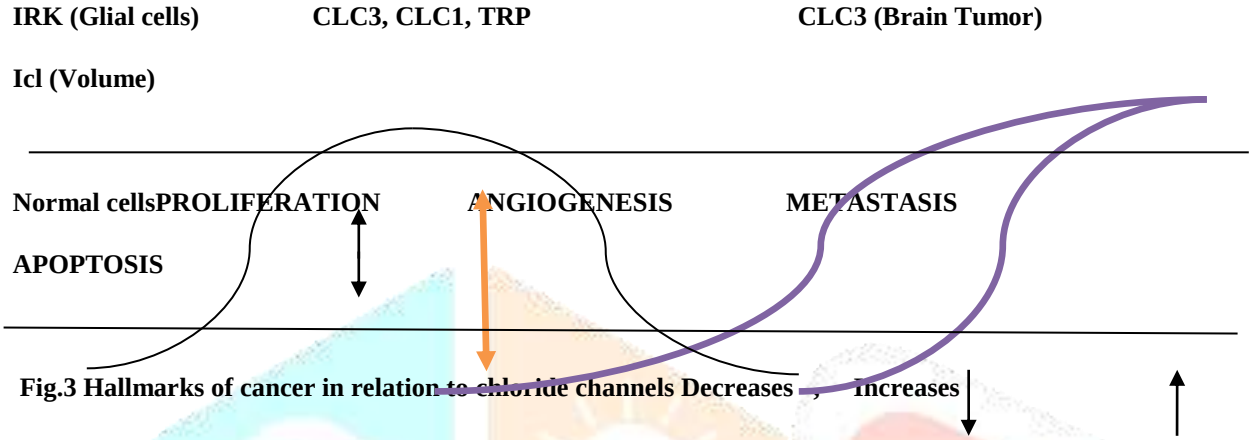

Fig.3 Hallmarks of cancer in relation to chloride channels

uring the stages of a tumor progression a normal cell goes through someincrease in proliferation and a decrease in apoptosis then increase in giogenesis and ultimately metastasis. When apoptosis stops then the tumor goes through a state of ischemia, to get more blood supply some branches vessels form the main vessels and that is called as angiogenesis [4,10,26]. Tumor is always bound by a protein called cadherin and after a time dherin becomes loose and the binding capacity for cadherin is lost and then tumor metastasizes to various places through haematogenous (sarcoma) and mphatic (Carcinoma) route. For example, Primary adenocarcinoma metastasizes to brain, Breast cancer Metastasizes to liver etc. Role of the chloride annels with respect to cancer first arose when the multidrug resistance protein (MDR glycoprotein) was linked to volume activated channel chloride erapy (VCCT) in cancer cells from patients undergoing chemotherapy [1,4].Since the chloride ion channels have several functions like modifying cell cle, causing apoptosis, causing cell adhesion and also cell motility [5] an increased unregulated cell proliferation, reduction of cellular physiological optosis, genomic instability, genetic variation, genetic mutation, increasing nuclear cytoplasm ratio, increasing mitotic activity and invasive and etastatic cancer are features of tumorigenesis and are also the hallmarks of cancer, as a result we can conclude that chloride ion channels play an portant role in cancer [4].

DNA Methylation of channel related to cancer [1,2,81,89]

ethylation consists of the addition of a methyl group on a nucleotidic base of the DNA through the action of DNA methyl transferase enzymes. In karyotic cell, only cytosine can be methylated at the 5' position.

int or frameshift type mutation of specific genes causes cellular transformation and genetic instability by which a normal cell is converted into a ncerous cell. Epigenetic mechanisms like DNA methylation is also involved in this conversion. This mechanism potentially targets the genes which are coding for ion channels. As their expression is up or down regulated in cancer and actively transcribed genes are associated with promoting **permethylation in DNA** causing cancer. In case of chloride channels, the genes responsible for their overexpression are ANOCTAMIN (ANO) genes om ANO1 to ANO10 located in the 11th chromosome. DNA methylation contributes to cancer in the following ways:

1) Hypomethylation of the cancer genome activates oncogenes like Abl-Bcr gene and causes genomic instability.
2) Hypermethylation of the tumor suppressor genes like p53 inactivates their transcription. This is observed in P53 in all type of cancers, RBinrertinoblastoma, BRCA1, BRCA2in breast cancer.
3) Methylation of CpG's causes binding of the chemical carcinogens.

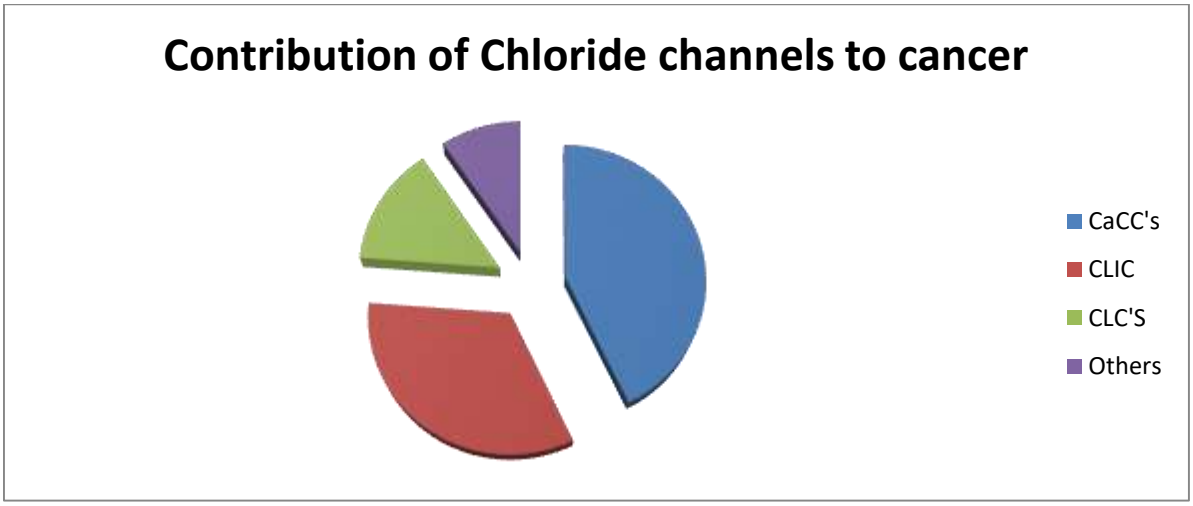

Fig.4. Contribution of chloride channels in cancer

Name of the Chloride ion channel	Tumor Type	Methylation Status
CFTR	Lung cancer, Non-small cell lung cancer, bladder cancer, hepatocarcinoma, breast cancer in MCF-7 cell line	Hyper
CLCA2	Breast Cancer	Hyper

Table. 1. Chloride channels and their alteration in different type of cancer .(Role of anoctamins in cancer and apoptosis Podchanart Wanitchakool,[1] Luisa Wolf,*et al.*,2014)

oles of the following Chloride Channels in Cancer

CFTR [4,5,91,92,93,94,95,96]

- Mutations of the CFTR gene affect the functioning of the channel which causecystic fibrosis. CFTR gene involved in multiple molecular pathways that control apoptosis and inflammation. Cancer is caused because of the modification in these genes and decrease in cell apoptosis. Pancreatic cell line has shown maximum amount of CFTR gene mutation. (Mc Williams et al 2010). Over expression of this gene is related with cervical cancer progression, CIN 4 shows the maximum amount of overexpression of CFTR gene. So the channel can be used as tumor marker and as therapeutic agent. (Peng et al 2012). It is found that mutations in the CFTR gene may have a protective function in tumors of lungs, melanoma, colon and breast (Padua R A et al 1997).
- Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene are common in white persons and are associated with pancreatic disease. That why the incidence and prevalence of cystic fibrosis and pancreatic disease like pancreatitis, CA pancreas are more in western countries. Carrying a disease-associated mutation in CFTR is associated with an increase in risk for pancreatic cancer. Those affected appear to be diagnosed at a younger age, especially among smokers. CFTR gene is present in most of the people but they are either functionally normal or they are in a inactive condition. But carcinogen like tobacco increase

the chance of mutation in the CFTR gene which increase the chance of CFTR related cancers by 20 to 30 fold. Clinical evidence of antecedent pancreatitis was uncommon among both carriers and noncarriers of CFTR mutations.

- Higher CFTR expression is closely associated with cervical cancer progression, aggressive behavior and poorer prognosis, indicating that CFTR may function as a good tumor marker, a prospective prognostic indicator and a potential therapeutic target for cervical cancer. So with the histopathology of cervical cancer we can also use CFTR expression to understand the prognosis of cervical cencer.
- The cystic fibrosis transmembrane conductance regulator (CFTR) holds an important role in retaining lung function, but its association with lung cancer is not clearly understood. Experiment has shown that genetic variations in the CFTR gene might modulate the risk of lung cancer.

Voltage gated chloride channels [69,70,71,72,73,74,75,]

CLC-3 a chloride channel plays an important role in volume regulation, cell migration and apoptosis. Volume changing inside the cell plays an important role in cancer progression. CLC-3 expression has been observed in human prostate cancer cells. Due to over expression of Bcl-2 onco-protein, an anti-apoptotic regulator that interferes with the volume, regulates anion channel and interferes with the cell volume causing its increase. Studies have shown that volume activated chloride channel is expressed in much higher amount in nasopharyngeal carcinoma than in normal cells. The activity of the volume activated chloride channel is one of the most important factors that regulate the passage of cells through G1 restriction point in the cell cycle, and chloride concentration controls cell proliferation.

CLC-3, CLC-2 and CLC-5, these three channels are expressed in glioma cell membranes.Reduction of cell tumor happens due to Glioma cell metastasis.

Calcium activated chloride channel [2,81,82,83,84,85,86]

CLCA-2 is expressed in trachea and lungs. It is shown that loss of CLCA-2 in breast cancer is associated with tumorigenicity and the number of CLCA-2 is down regulated in colon cancer. Another member of the calcium activated chloride channel family is the anoctamin-1(Ano1), which is highly expressed in the gastro-intestinal tract (GI tract). It is proved that ANo-1 is highly expressed in gastric tumors like adenocarcinoma of stomach and linitica plastic or diffuse gastric tumor and it maintains cell proliferation by regulating chloride ion entry at the G1/S transition of the cell cycle.

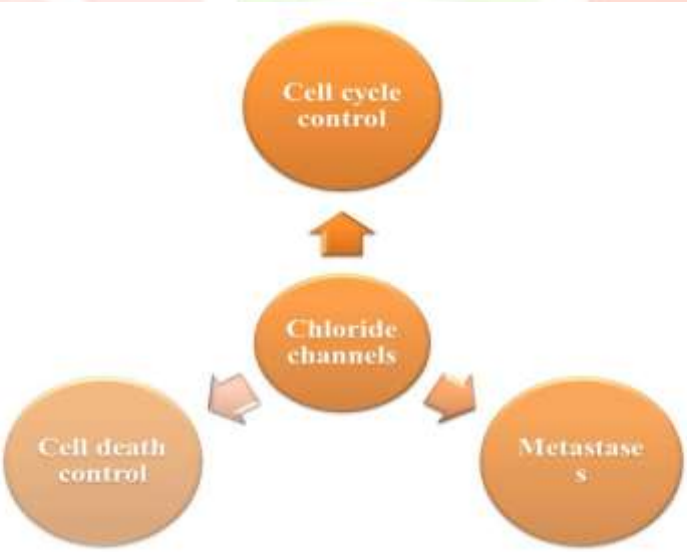

Fig. 5.Chlorode and cancer phenotype

Contribution of ANOCTAMINS in cancer(Luisa W, KulkarniS*eta.,l* 2014) [2,15,16,18,81,82,87,89,90,160]

Anoctamins are chloride currents activated by an increase in intracellular Ca2+ (CaCC). CaCCsare present primarily in proliferating cells in culture and various types of cancer cells. ANO1 has been found in different malignant tumors. Apart from ANO1, other members of the anoctamin family were also correlated with cell proliferation and cancer development, like ANO5 (TMEM16E), ANO7 (TMEM16G) and ANO9 (TMEM16J). Anoctamins have tumor-specific functions, or may support cell proliferation and possible development towards malignancy. Compared to Ano1, other anoctamins are less known and their effect is poorly known to us. Moreover, some anoctamins, like ANO6, may even promote cell death, rather than growth. So ANO6 can be used in the treatment measures of cancers. Yang *et al* proposed the new name "anoctamin" or Ano (anion+octa=8) and this name is now the official HUGO nomenclature and has replaced or changed Tmem16 in Genebank. Tmem16A is now Ano1, Tmem16B is Ano2, and so-on, except the letter I is skipped in the Tmem16 nomenclature so Tmem16I has no existence and so that Tmem16J is Ano 9 and Tmem16K is Ano10.

Individual details discussion of anoctamins in different types of cancer

ANOCTAMIN FAMILY	Expression found in Cancer subtypes
ANO1	GIST, pancreas, prostate, breast, colon, gastric, blastoma, esophagus, lungs, liver, ovary, salivary gland
ANO2	Endometrium
ANO5	Colorectal, thyroid
ANO6	Myoblast proliferation
ANO7	Prostate, breast
ANO9	Pancreas, colorectal

Table .2 .ANO family and their different expression (DNA methylation of channel-related genes in cancers.(Ouadid-Ahidouch H et al.,2015)

ANO-1

The role of ANO-1 in cancer will be discussed briefly in this section.

1. The calcium activated chloride channel ANO-1 is over expressed in many tumors, one of the most frequently amplified chromosomal regions in human cancer. ANO1 is amplified and expressed in human breast cancer cell line about 15% higher than a normal cell.
2. It is shown that ANO1 plays an important role in metastasis of cancer. During hypotonic cell swelling it gets activated, and contributes to regulatory volume decrease which requires a rise in intracellular calcium ion, resulting in osmotic loss of cells which cause cell shrinkage and resulting in decrease in cell volume.
3. Gastrointestinal stromal (GIS) tumors are most common mesenchymal tumors of the GIT. Due to overexpression of ANO1, the GIS takes place..
4. It is also frequently over expressed in head and neck squamous cell carcinoma.
5. It gets highly amplified in prostate cancer cells, regulating their migration and expression.
6. Spatial and temporal regulation of the activity at each end create an unequal distribution of membranous, cytoskeletal and cytoplasmic contents that induces a highly polarized and motile shape suitable for metastasis.
7. In oral and head and neck squamous cell carcinomas, amplification of the Ano1 locus is related with a poor outcome. Ano1 expression is significantly increased in patients with a propensity to develop metastases. Given the role that Cl- channels play in cell proliferation and migration, it is possible that Ano1 overexpression provides a growth or metastatic advantage to cancer cells. Supporting the role of Ano1 in metastasis is that overexpression of Ano1 stimulates cell movement. In contrast, silencing of Ano1 decreases cell migration and treatment of cells with Calcium channel blocker like amlodipine, Clinodipine, Nimodipine, Diltiazam, Verapamil has a similar effect.

ANO1 and Sonic Hedgehog

Interesting links exist between ANO1 and the sonic hedgehog signaling pathway. It controls many aspects of development and also regulates cell growth and differentiation in adult tissues. It controls cell growth and tissue morphogenesis. It is also active in embryonic cancers like basal cell carcinoma (BCC) of the skin and also during epithelial to mesenchymal transition. Hedgehog expression is upregulated in the neoplastic tissues, while suppression by cyclopamine shows apoptosis.

ANO1 and mTOR pathway

In mouse model ANO1 expression was found to be regulated by tumour suppressor gene calledadenomatosis polyposis coli (APC). ANO1 is expressed in mouse ileum, proximal and distal colon (PC and DC), but its expression is largely attenuated in APC gene in case of mice. APC-min mice demonstrated reduced tumour suppressor activity, resulting in upregulation of mTOR, leading to numerous large intestinal polyps(polyposis) and neoplastic cells.mTOR is also seen in case of metastatic hormone resistant breast cancer. mTOR upregulations cause over proliferations of cells and that is no longer a normal cell. That time it is a neoplastic cell or commonly called as cancer cell. It was founded out that due to a decrease in ANO1 expression APC-min mice developed polyps in distal colons.

ANO1 and Volume Regulated Anion Channel (VRAC)(Luisa W, Kulkarni S *et al*.,2014)

All living cells are able to maintain a constant cell volume. According to a general concept, regulatory volume decrease (RVD) prevents cell swelling and necrotic cell death, while regulatory volume increase (RVI) prevents cell shrinkage and apoptotic cell death.The volume regulated or swelling activated anion channels(VRAC) are activated during RVD. Excessive activation of VRAC may support apoptotic cell death, while its upregulation leads to cellular resistance towards anti-cancer drugs. So we have to prevent upregulation of VRAC and use VRAC in cancer treatment. Recent experiments suggest that ANO1 also contribute to volume activated whole cell currents, which may indicate a possible functional link between anoctamins and VRAC.

REGULATION OF ANO1 BY HISTONE DIACETYLASE AND ITS CLINICAL SIGNIFICANCE (Luisa W, Kulkarni S *et al*.,2014)

Histone deacetylases (HDACs) are the main element in the dynamic regulation of genes controlling cellular proliferation and differentiation during normal development and carcinogenesis. (Jenuwein T, Allis CD. 2001). Some of the anti-cancer treatments are based on the inhibition of HDAC. HDAC inhibitors promote expression of p21 in breast cancer cells. This P21 inhibits the action of cyclin D1. HDAC inhibitors may therefore also be useful for the treatment of that squamous cell carcinoma mainly in lungs, esophagus and cervix, that show overexpression of Ano1 and concomitant activation of cyclin D1. Smoking can show the increase in HDAC inhibition, so smoking increase squamous cell lung cancers where a keratin pulse and mitotic body is seen in the histopathological slides.

ANO-2

Endometrial cancer is one of the most commoncancer of the female reproductive system which shows dysmenorrhea and vaginal bleeding after menopause. It is observed that expression of ANO2 is increased in patients with endometrial cancer. This gene is involved in RNA splicing, covalent chromatin modification, histone modification and organelle fission.

ANO-4

Researchers have concluded that its expression is seen in HeLa, TERT 166, SK-MEL 30 cell lines.HeLa and MET 30 are the most common cell lines in the clinical settings. Its expression is also seen in cancers of colorectal, breast, prostate, lungs and colon cancer.

ANO-5

Dysregulation of ANO5 participates in tumorigenesis and progression. However, the exact role of anoctamin5 (ANO5), in thyroid cancer is still not clarified. Studies found that the expression levels of ANO5 were downregulated in thyroid cancer compared to adjacent normal tissue by mining the public GEO database. Subsequently demonstrated that the expression levels of ANO5 was significantly downregulated in 69.5% (57/82) clinical thyroid cancer tissues using real-time PCR (rt PCR) assay. Moreover, western blot assay also showed that ANO5 was downregulated in papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC) compared to adjacent noncancerous tissues. It is observed that ANO5 also plays an important role in osteoblastoma (most common tumor in brain) and sarcolemma.

ANO-6(Szteyn K, Schmid E et al 2012)

1. Ano6 has been characterized as a calcium-activated chloride channel. However, Chloride (Cl-)channel and scramblase activity were shown to be independent. It is demonstrated recently that Ano6 is activated during cell swelling and by pro-apoptotic stimuli and therefore contributes to both RVD as well as apoptotic volume decrease. Thus, anoctamins (Ano) can also be regarded as a novel

family of regulators of cell proliferation and apoptosis, which may be of particular relevance during development, activation of immune cells such as lymphocytes, APCs and in particular types of cancer.

2. Pro-apoptotic Cl^- currents have been activated in cells overexpressing ANO6. The bee venom melittin is a potent activator of anoctamins, which stimulates phospholipase A2 (PLA_2). Melittin is widely used as anti-cancer therapy, and PLA_2-dependent activation of metalloproteinase is essential for this effect. Anoctamins are also activated through reactive oxygen (0-) species and by lipid peroxidation. This may lead to inflammation and proliferation, ion secretion and ferroptosis, the anoctaminparalogue being activated, and the strength of peroxidation. Ferroptosis is induced by accumulation of intracellular iron, and is distinct from apoptosis, necrosis, and other forms of regulated cell death. So Ferroptosis is a key in the cancer treatment.Ferroptosis is triggered by an increase in reactive oxygen species (ROS) and an overwhelming lipid peroxidation that ultimately leads to cell death by disintegration of the plasma membrane.

   Cell death can be induced in cancer cells by activation of ANO6 through melittin-induced PLA_2 or through lipid peroxidation This suggests a new potential therapeutic approach to inhibit growth of cancer. Lipid peroxidation and ferroptosis-induced cell death was proposed earlier as a mechanism to destroy cancer cells. However, the ROS buffer capacity is typically quite high in cancer cells, which will antagonize lipid peroxidation. ROS levels could be enhanced to exceed the antioxidant defence of cancer cells.
3. Anoctamin 6 (ANO6), a member of the anoctamin chloride channel family, is localized in the primary cilium of renal epithelial cells in vitro and in vivo. ANO6 was not essential for cilia formation and had no effect on in vitro cyst expansion. Knockdown of ANO6 impairs cyst lumen formation of MDCK cells in three-dimensional culture. In the absence of ANO6, apoptosis was reduced and epithelial cells were incompletely removed from the center of cell aggregates, which form in the early phase of cystogenesis and neoplastic cell formation. In line with these data, ANO6 is highly expressed in apoptotic cyst epithelial cells of a human polycystic kidney. So mutation in Ano6 can cause Autosomal dominant polycystic kidney disease (ADPKD) and Autosomal recessive polycystic kidney disease(ARPKD) These data identify ANO6 as a cilium-associated protein and suggest its functional relevance in cyst formation mostly in the kidney.

ANO-7

1. A gene NGEP (Isoform of ANO7)is expressed only in prostate cancer and normal prostate. The two NGEP transcripts are 0.9 kb and 3.5 kb in size and are generated by a differential splicing event. The short variant (NGEP-S) is derived from four exons and encodes a 20-kDa intracellular protein. The long form (NGEP-L) is derived from 18 exons and encodes a 95-kDa protein that is predicted to contain seven-membrane-spanning regions. In situ hybridization shows that NGEP mRNA is localized in epithelial cells of normal prostate and prostate cancers. Because of its selective expression in prostate cancer and its presence on the cell surface, NGEP-L is a promising target for the antibody-based therapies of prostate cancer.
2. NGEP protein is widely expressed in low-grade to high-grade prostate adenocarcinomas as well as benign prostate tissues, and the intensity of expression is inversely proportional to the level of malignancy. NGEP could be an attractive target for immune-based therapy of prostate cancer patients as an alternative to the conventional therapies.
3. D-TMPP, an isoform of ANO7 gene is widely expressed in human prostate cancer.
4. The D-TMPP-mRNA encodes a putative seven-span transmembrane protein of 883 amino acids and is selectively overexpressed in prostate tissue. D-TMPP transcripts were detected in all analyzed pairs of malignant and nonmalignant prostate tissues. In the androgen-dependent PCa cell line LNCaP, D-TMPP was upregulated by methyltrienolone. Hence the novel prostate-restricted molecule D-TMPP is widely expressed in prostate cancer tissues.

ANO-9

1. ANO9 is overexpressed in pancreatic cancer cells, and its role in the pathogenesis of pancreatic cancer was evaluated using an integrated in vitro and in vivo approach. The ANO9 mRNA and protein levels were increased in pancreatic cancer-derived cells. Exogenous expression of ANO9 in PANC-1 cells significantly increased cell proliferation in cell cultures and in mice. In contrast, knockdown of ANO9 in AsPC-1, BxPC-3, and Capan-2 cells strongly inhibited cell proliferation. Mechanistic analysis suggested that physical association of ANO9 with epidermal growth factor receptor (EGFR) underlies ANO9-induced cell proliferation. Knockdown of ANO9 augmented the effects of the EGFR inhibitor and the cytotoxic agent on pancreatic cancer cell proliferation. In addition, high ANO9 expression is a poor prognostic factor in patients with pancreatic cancer. The ANO9 appears to be a clinically useful prognostic marker for pancreatic cancer and a potential therapeutic target.

2. Lower expression of ANO9 messenger RNA (mRNA) was frequently detected both in CRC tissues with recurrence and metastasis-derived cell lines. Compared with matched nontumorous tissues, lower expression of ANO9 protein was observed in tumors, which was significantly correlated with tumorigenesis. Moreover, investigation of clinical CRC specimens showed that ANO9 were markedly overexpressed in metastatic tissue compared with primary tissue. Decreased expression of ANO9 was correlated with poor prognostic outcomes.

CLIC-1(Peretti M, Angellini M *et al*.,2015)

[1,6,7,8,25,38,39,43,45,47,48,52,54,56,60,61,63,64,99,100,101,108,109,113,134,135]

1. CLIC-1 proteins level are expressed in higher amounts in breast cancer, gastric cancer, gallbladder metastasis, colon cancer, nasopharyngeal carcinoma, ovarian cancer, liver cancer and gliomas. CLIC-1 contributes to promoting migration and invasion. In liver cancer, the down regulation of CLIC-1 enhances proliferative activity, increases the ratio of cells entering G2/M phase and decreases the percentage of apoptosis.
2. The function of CLIC-1 in LOVO cells- a human colon adenocarcinoma cell line is characterized by a high metastatic potential of colon cancer cells. Migration and invasion of colon cancer cells are inhibited when the expression of CLIC-1 is down regulated. The inhibition of CLIC-1 also decreases the ROS production leading to decreased cell migration. The metastasis of colorectal cancer is one of the most common causes of death in the world. The human colon cancer cell lines LOVO and HT29 as model systems to determine the role of the chloride intracellular channel 1 (CLIC1) in the metastasis of colonic cancer. In the present study, it was found that regulatory volume decrease (RVD) capacity is markedly up-regulated in LOVO cells, which are characterized by a high metastatic potential. Functionally suppressing CLIC1 using the chloride intracellular channel 1 blocker inhibits RVD and decreases the migration and invasion of colon cancer cells. CLIC1 modulates the metastasis of colon cancer through its RVD-mediating chloride channel function.
3. Another important role of CLIC-1 is associated with the development of glioblastoma, the most aggressive and frequent brain tumor. CLIC-1 is highly expressed in glioblastomas and both mRNA and protein levels are increased in higher grade in comparison to lower grade brain tumors. In these tumors the bulk of malignant cells are generated by a fraction of self renewing cancer stem cells after they are degraded with chemotherapy.
4. Chloride intracellular channel 1 (CLIC1) is expressed in many human tissues and has been reported to be involved in the regulation of cell cycle, cell proliferation, and differentiation. It plays a role in human hepatic tumor. The aim of this study is to investigate the clinicopathological significance and expression pattern of CLIC1 in human primary hepatic tumors. Increased CLIC1 protein expression is associated with clinicopathological factors and a poor prognosis of hepatic tumors, and suggests that CLIC1 might represent a valuable prognostic marker for human hepatic tumors.
5. It is observed that CLIC-1 regulates colon cancer cell migration and invasion through reactive oxygen species (ROS) pathway.
6. It is observed that CLIC-1 plays an important role in development of sarcoma as well. Sarcomas are rare forms of cancer with a high clinical need that develop in connective tissue, such as muscle, bone, nerves, cartilage, and fat. The outcome for patients is poor, with surgery and postoperative radiotherapy, the standard treatment for patients. An immunohistochemsitry test is being performed along with proteome screening for this confirmation. A proteome screen using tandem mass tag isobaric labeling on three high-grade undifferentiated pleomorphic sarcoma biopsies from different tissue site isperformed. It was identified that the commonly dysregulated proteins within the three sarcomas is the CLIC1.
7. PA28β is a subunit of proteasome activator of PA28; PA28β is involved in the invasiveness and metastasis of gastric adenocarcinoma (GA). The invasive abilities of gastric cancer cells were enhanced when PA28β being down-regulated, and are inhibited when PA28β is overexpressed. Down-regulation of CLIC1 by RNA interference is able to markedly inhibit cell invasion of PA28β, PA28β can enhance tumor invasion and metastasis through up-regulation of CLIC1.
8. CLIC1 and TPD52(Tumor protein D52) are significantly up-regulated in all cases of colorectal cancer investigated, irrespective of localization, pTNM stage and grade of colon cancer highlighting their potential to serve as new biomarkers.
9. Gastric cancer is the second most common cancer worldwide. Identification of biomarkers is essential to improve patient survival. Fifty aberrantly expressed proteins were identified using a mass spectroscopy method, 2-DE combined with MALDI TOF MS and were grouped based on their function.Expression of chloride intracellular channel 1 (CLIC1) is significantly up-regulated in most of the gastric patients. Elevated CLIC1 expression is strongly correlated with lymph node metastasis, lymphatic invasion, perineural invasion, and pathological staging.
10. CLIC1 is a critical factor in the development of lymphatic metastasis.

CLIC-4 (Peretti M, Angellini M et al 2015)

[1,9,22,25,40,41,42,50,51,53,57,58,60,62,100,112,113,114,115,116,117,118, 121,123,127,128]

1. CLIC-4 expression is found in multiple human epithelial cancers. The loss of CLIC-4 in tumor cells and the gain of its expression in tumor stroma are common traits of many human malignant cancers. CLIC-4 expression was found to be diminished in the bladder cancer, uterine leiomyoma, glioma, melanoma, colon cancer stem cells, ovarian cancer, cancer of the oesophagus, cervix. CLIC-4 also participates in angiogenesis where it is required for endothelial tube formation through reduction of pH of vacuoles during angiogenesis.
2. Cancer stroma has a profound influence on tumor development and progression. The conversion of fibroblasts to activated myofibroblasts is a hallmark of reactive tumor stroma. Among a number of factors involved in this conversion, transforming growth factor (TGF)-β has emerged as a major regulator. CLIC4, an integral protein in TGF-β signaling, is highly upregulated in stroma of multiple human cancers, and overexpression of CLIC4 in stromal cells enhances the growth of cancer xenografts. Overexpression of CLIC4 increases tumor cell migration and invasion in a TGF-β-dependent manner and promotes epithelial to mesenchymal

transition, indicating that high stromal CLIC4 serves to enhance tumor invasiveness and progression. Thus, CLIC4 is significantly involved in the development of a nurturing tumor microenvironment by enhancing TGF-β signaling. Targeting CLIC4 in tumor stromashould be considered as a strategy to mitigate some of the tumor enhancing effects of the cancer stroma.

3. It is observed that CLIC4, ERp29, and Smac/DIABLO integrated into a novel panel based on cancer stem-like cells in association with metastasis stratify the prognostic risks of colorectal cancer. Prediction of risks with molecular markers will benefit clinicians to make decisions of individual management with postoperative colorectal cancer patients.
4. CLIC-4 also sometimes plays a role in lung cancer. The restoration of CLIC4 in lung cancer cell lines in which CLIC4 expression was reduced attenuated their growth activity. The immunohistochemical expression of the CLIC4 protein is weaker in primary lung cancer cells than in non-tumorous airway epithelial cells. These suggest that the alteration in CLIC4 could be involved in restrictedly the development of a specific fraction of lung adenocarcinomas.
5. Stromal myofibroblasts, activated by crosstalk signaling between the tumour and stroma, play a critical role in tumour development and progression. Chloride intracellular channel 4 (CLIC4) may functionally import for tumour stromal fibroblast-to-myofibroblast transdifferentiaton.

CLC-3

[11,12,14,31,32,33, 34,37,69,71,72,73,77,144,145,147,149,150,151,152]

1. Brain cells are postmitotic, small populations of progenitor or stem cells can divide throughout life. These cells are believed to be the most likely source for primary brain malignancies including gliomas. Such tumors share many common features with nonmalignant glial cellsbut because of their insidious growth cancers form that are typically incurable. In studying the growth regulation of these tumors, it was discovered that glioma cell division is preceded by a cytoplasmic condensation and by means of ion excretion and the associated obligatory water loss;glioma cells can change shapes and undergo extensive migration and invasion. Glioblastomamultiforme is the most common and lethal primary brain cancer in adults. Tumor cells infiltrate the brain via diffusion and makes surgical and radiation treatment challenging. The invasion of glioma cells into the brain is facilitated by the activity of a voltage gated chloride channel by dynamic regulation of cell volume. ClC-3 is highly expressed on the plasma membrane of human glioma cells where its activity is regulated through phosphorylation via Ca^{2+}/calmodulin- dependent protein kinase II (CaMKII). It has been observed that in ClC-3-expressing cells, inhibition of CaMKII reduced glioma invasion to the same extent as direct inhibition of ClC-3.

   Blockage of a single ClC, however, is not sufficient to achieve complete inhibition of glioma cell invasion, as along with CLC-3 , CLC-2 ,4,5,6, and 7 are also overexpressed, suggesting that any future therapy should be targeted at pharmacological blockage of multiple ClCs.
2. Chloride current and apoptosis are found to be induced by paclitaxel and inhibited by chloride channel blockers. Paclitaxel-activated current possesses similar properties to volume-activated chloride current. After ClC-3 is knocked-down by ClC-3-siRNA, hypotonicity-activated and paclitaxel-induced chloride currents are decreased, indicating that the chloride channel involved in paclitaxel-induced apoptosis is ClC-3. In early apoptotic cells, ClC-3 is up-regulated significantly; over-expressed ClC-3 is accumulated in cell membrane to form intercrossed filaments, which are co-localized with α-tubulin. These suggest that ClC-3 is a critical target of paclitaxel.
3. It is observed that suppression of CLC-3 inhibited the migration of CNE-2Z cells, a nasopharyngeal carcinoma cell line. ClC-3 is a component or regulator of the volume-activated Cl^- channel. ClC-3 regulates CNE-2Z cell migration by modulating cell volume.
4. Cervical carcinoma is a major gynecological cancer and causes cancer-related deaths in worldwide, the latent pathogenesis and progress of cervical cancer is still under research. ClC-3 is an important promoter for aggressive metastasis of malignant tumors.
5. Breast cancer tissues collected from patients shows an increase in ClC-3 expression. Knockdown of ClC-3 inhibits the secretion of insulin-like growth factor (IGF)-1, cell proliferation, and G1/S transition in breast cancer cells. In the mouse xenograft model of human breast carcinoma, tumor growth is significantly slower in animals injected with ClC-3-deficient cells compared with the growth of normal human breast cancer cells.
6. In cultured human osteosarcoma (OS) cells, it is observed that demonstrated that insulin-like growth factors (IGF-1)-induced MG-63 and 143B human OS cells proliferation are consistent with increasing ClC-3 expression, and ClC-3 is up-regulated in cells with high metastatic potency. Blockade of ClC-3 greatly suppresses the phosphorylation activation of Akt/GSK3β. It has also been found that blockade of ClC-3 effectively down-regulated the expression of cyclin D1 and cyclin E, and caused activation of p27(KIP) and p21(CIP). The synthesized effects on these proteins which play a major role in cell cycle regulation bring about G0/G1 cell cycle arrest in MG-63 cells, and finally abrogate the cell proliferation. Besides, ClC-3 deletion attenuates OS cell migration via down-regulation the expression of MMP-2 and MMP-9. Such information suggests that ClC-3 might be a potential target for anti-OS.
7. Acid-activated chloride currents have been reported in several cell types and may play important roles in regulation of cell function. Activation of the acid-induced chloride current and the possible candidates of the acid-activated chloride channel were investigated in human nasopharyngeal carcinoma cells (CNE-2Z). A chloride current is activated when extracellular pH was reduced to 6.6 from

7.4. However, a further decrease of extracellular pH to 5.8 inhibits the current. The current is weakly outward-rectified and is suppressed by hypertonicity-induced cell shrinkage and by the chloride channel blockers 5-nitro-2-3-phenylpropylamino benzoic acid (NPPB), tamoxifen, and 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid disodium salt hydrate (DIDS). The permeability sequence of the channel to anions is I(-) > Br(-) >Cl(-) >gluconate(-).ClC-3 are strongly expressed in CNE-2Z cells. Knockdown of ClC-3 expression with ClC-3 small interfering (si) RNA prevents the activation of the acid-induced current.

CLCA-2 [4,15,16,17,18,65,66,81,82,83,84,85,86,87,90,105]

1. Expression of hCLCA2 (H=human gene) in normal mammary epithelium is consistently lost in human breast cancer and in all tumorigenic breast cancer cell lines. Re-expression of hCLCA2 in human breast cancer cells abrogates invasiveness of Matrigel (BD Biosciences-Labware, Bedford, MA, USA) in vitro and tumorigenicity in nude mice, implying that hCLCA2 acts as a tumour suppressor in breast cancer. It is expressed in normal breast epithelium but not in breast tumors of different stages of progression. Northern analysis of nontransformed and transformed breast epithelial cell lines revealed CLCA2 expression in the nontransformed cell line MCF10A and the nontumorigenic cell line MDA-MB-453, whereas all tumorigenic cell lines were negative (MDA-MB-231, MDA-MB-435, MDA-MB-468, and MCF7).
2. CLCA2 may function as a tumor suppressor in colorectal cancer like that of in breast cancer.
3. hCLCA2 is frequently down-regulated in breast cancer and is a candidate tumor suppressor gene. The hCLCA2 gene is strongly induced by p53 in response to DNA damage. Adenoviral expression of p53 induces hCLCA2 in a variety of breast cell lines. p53 binds to consensus elements in the hCLCA2 promoter and mutation of these sites abolishes p53-responsiveness and induction by DNA damage. Adenoviral transduction of hCLCA2 into immortalized cells induces p53, CDK inhibitors p21 and p27, and cell cycle arrest by 24 hours, and caspase induction and apoptosis.

otential therapies by targeting chloride channels

113,114,121,123,126,131,132,137,138,139,140,141,146,147,152,155,156,157,158,159]

low the potential therapies for treating cancer using chloride ion channel will be discussed.

- ❖ It has been observed that increase of CLIC-4 expression in cells by transduction of recombinant CLIC-4 causes apoptosis. Reduction of CLIC-4 and several other CLIC family members by expressing a doxycycline regulated CLIC-4 antisense also causes apoptosis in squamous cancer cell lines. It is observed that in mice expressing antisense CLIC-4 in tumors derived from transplanting these cells cause apoptosis. Synergistic administration of Tumor necrosis factor alpha influences tumor inhibitory action of this protein.
- ❖ Cell swelling activates outer movement of Cl- ions in endothelial cells that are controlled by the volume regulated anionic channels. Angiogenesis in endothelial cells are arrested in the presence of compound that potentially blocks the VRAC's at the endothelium.
- ❖ It has been discussedearlier that CLC-3 plays an important role in nasopharyngeal carcinoma cells and helps in their metastasis. It is observed that suppression of CNE-2Z cells found in these cancer cell lines inhibits cancer growth.
- ❖ CFTR is expressed in a wide variety of epithelial cells including the prostate cancer cell lines. Low expression of the CFTR polymorphism and mutations by using an antisense strand could reduce the risk of prostate cancer.
- ❖ Ca2+ activated chloride channel (CaCC) is expressed in higher amounts in epithelial cells and functions to maintain epithelial secretion and cell volume for maintenance of tissue homeostasis in proliferation, apoptosis. ANO1 plays an important role in metastasis of prostate cancer. Evidences have shown that ANO1 serves as a biomarker and the inhibition of it's over expression could be used as a potential therapy against prostate carcinoma.
- ❖ It is observed that CLIC-4 expression is increased during TNF alpha induced apoptosis in human osteosarcoma cell lines. Inhibition of NFkB results in increase of TNF alpha mediates apoptosis with a decrease of CLIC-4. Cell lines inducing a CLIC-4 antisense protein reduces the expression of other CLIC members and causes apoptosis in these cells and hence inhibits tumor growth.

- Glioblastoma is the most prevalent and malignant form of brain tumor with no potential therapeutic approach discovered yet. But there are some areas of therapeutic research for glioma treatment using cancer stem cells, and targeting other tumor initiating cells.
- Metformin a drug for type II diabetes has proved to possess a potential anti tumor activity. It has been shown that metformin shows anti-tumor activity in breast cancer models and also in glioblastomas.
- CLIC-1 ion channel is active during the G1-S phase transition of the cell cycle. Metformin drug administration induces G1 arrest of glioblastoma stem cells. CLIC-1 is the direct target for metformin in human glioblastoma cells. It expresses anti-proliferative effect in tumors.
- Epidemiological and preclinical studies propose that metformin, a first-line drug for type-2 diabetes, exerts direct antitumor activity. Although several clinical trials are ongoing, the molecular mechanisms of this effect are unknown. CLIC1is a direct target of metformin in human glioblastoma cells. Metformin exposure induces antiproliferative effects in cancer stem cell-enriched cultures, isolated from three individual WHO grade IV human glioblastomas. These effects phenocopy metformin-mediated inhibition of a chloride current specifically dependent on CLIC1 functional activity. CLIC1 ion channel is preferentially active during the G1-S transition via transient membrane insertion. Metformin inhibition of CLIC1 activity induces G1 arrest of glioblastoma stem cells. This effect was time-dependent, and prolonged treatments caused antiproliferative effects also for low, clinically significant, metformin concentrations. The lack of drugs affecting cancer stem cell viability is the main cause of therapy failure and tumor relapse. CLIC1 is not only a modulator of cell cycle progression in human glioblastoma stem cells but also as the main target of metformin's antiproliferative activity, paving the way for novel and needed pharmacological approaches to glioblastoma treatment.
- We know that angiogenesis is one of the hallmarks of cancer. Preventing it will inhibit tumor growth. Osmotic cell swelling activates an outwardly rectifying Cl^- current in endothelial cells that is mediated by volume-regulated anion channels (VRACs).Serum-induced proliferation of endothelial cells is arrested in the presence of compounds that potently blocks the endothelial VRACs. Experiment was done by using four chemically distinct VRAC blockers [5-nitro-2-(3-phenylpropylamino)benzoic acid] (NPPB), mibefradil, tamoxifen, and clomiphene - on several models of experimental angiogenesis. VRAC blockers are potent inhibitors of angiogenesis and thus might serve as therapeutic tools in tumor growth.
- TM-601 binds to malignant brain tumor cells with high affinity and does not seem to bind to normal brain tissue. Preclinical studies suggest that iodine-131 (^{131}I) -TM-601 may be an effective targeted therapy for the treatment of glioma.
- Malignant gliomas, especially glioblastomamultiforme, are the most widely distributed and deadliest brain tumors because of their resistance to surgical and medical treatment. Research of glioma-specific bioconjugates for diagnosis and therapy developed rapidly during the past several years. Many studies have demonstrated that chlorotoxin (CTX) and ButhusmartensiiKarschchlorotoxin (BmK CT) specifically inhibited glioma cells growth and metastasis, and accelerated tumor apoptosis. The bioconjugates of CTX or BmK CT with other molecules have played an increasing role in diagnostic imaging and treatment of gliomas. CTX-based bioconjugates have achieved great success in phase I/II clinical trials about safety profiles.
  CTX and CTX-like peptide (BmK CT) represent novel and exciting platforms for glioma imaging and therapy due to the major advantages as follows: 1) small and condensed structure; 2) feasibility of artificial synthesis and the readily modified chemical structure with a tyrosine residue conjugating iodine or other molecules covalently; 3) rapid diffusion into tumor parenchymas and ability to penetrate the BBB; 4) slow elimination through the metabolism with a longer imaging time due to intracellular binding with glioma cells; 5) derivation from an invertebrate, being not rejected by human tissue, the absence of intimate toxicity without binding to normal tissue and cells; 6) antitumor activity in inhibiting tumor invasion and metastasis; and 7) antiangiogenic effects.
- Luteolin potentially inhibits ANO1 chloride channel activity in a dose dependent manner with an IC50 value of 9.8 micromolar. Down regulation of ANO1 by luteolin is a potential mechanism for anti-cancer effect.
- ANO1 inhibitors like CaCCinh-A01 and T16Aing-A01 could be used as a potential treatment against cancer.
- Idebenone, a synthetic analogue of coenzyme Q10 completely blocked ANO1 activity and thus it results in inhibition of cancer cell growth.
- Use of histone acetyl transferases and histone deacetylases (HDACs) is the key element in the dynamic regulation of genes controlling cellular proliferation and differentiation. A number of anticancer treatments are based on the inhibition of HDAC'S. HDAC inhibitors are used in the treatment of cancers that show over expression of ANO1 genes. Valproic and butyric acid are broad non selective inhibitors of HDAC, trichostatin selectively suppresses ANO1 expression.
- The mTOR inhibitor rapamycin increases ANO1 expression in both proximal and distal colon. The inverse relation between low ANO1 levels and upregulation of mTOR suggests that ANO1 may be inhibitory on proliferation of mouse intestinal epithelial cells. Treatment of neoplasia with mTOR inhibitor rapamycin reduced proliferation and induced expression of ANO1.
- Using nanomolar concentrations of the recently identified potent ANO1 inhibitor niclosamide, which has a number of additional anti-cancer effects. Nevertheless, other ANO1-inhibitors also blocked cell proliferation and cancer growth. Niclosamide is a FDA-approved drug and was shown to inhibit Notch signaling, a pathway that is well known to participate in tumorigenesis. In a number of reports, additional antineoplastic mechanisms of niclosamide have been described.
- Lowering the expression of CFTR polymorphs may contribute to a reduced risk of prostate cancer as observed in Chinese Han population.

- CLIC4 is a p53 and tumor necrosis factor α (TNFα) regulates intracellular chloride channel protein that localizes to cytoplasm and organelles and induces apoptosis when overexpressed in several cell types of mouse and human origin. CLIC4 is elevated during TNFα-induced apoptosis in human osteosarcoma cell lines. In contrast, inhibition of NFκB results in an increase in TNFα-mediated apoptosis with a decrease in CLIC4 protein levels. Cell lines expressing an inducible CLIC4-antisense construct that also reduces the expression of several other chloride intracellular channel (CLIC) family proteins were established in the human osteosarcoma lines SaOS and U2OS cells. Reduction of CLIC family proteins by antisense expression caused apoptosis in these cells. Moreover, CLIC4-antisense induction increased TNFα-mediated apoptosis in both the SaOS and U2OS derivative cell lines without altering TNFα-induced NFκB activity. Reducing CLIC proteins in tumor grafts of SP1 cells expressing a tetracycline-regolated CLIC4-antisense substantially inhibited tumor growth and induced tumor apoptosis. These suggest that CLIC proteins could serve as drug targets for cancer therapy, and reduction of CLIC proteins could enhance the activity of other anticancer drugs.
- Reactivation and restoration of CLIC4 in tumor cells or the converse in tumor stromal cells could provide a novel approach to inhibit tumor growth.
- The reduced expression of CLIC4 could further down-regulate MMP9 and result in the suppression of invasion in cancer cells treated with Photodynamic therapy (PDT) . These results provide an insight into a new mechanism by which PDT affects the metastatic potential of cancer cells through down-regulation of MMP9 by CLIC4.
- Alterations of signal transduction pathways leading to uncontrolled cellular proliferation, survival, invasion, and metastases are hallmarks of the carcinogenic process. The phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and the Raf/mitogen-activated and extracellular signal-regulated kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathways are critical for normal human physiology, and also commonly dysregulated in several human cancers, including breast cancer (BC). In vitro and in vivo data suggest that the PI3K/AKT/mTOR and Raf/MEK/ERK cascades are interconnected with multiple points of convergence, cross-talk, and feedback loops. Raf/MEK/ERK and PI3K/AKT/mTOR pathway mutations may co-exist. Inhibition of one pathway can still result in the maintenance of signaling via the other (reciprocal) pathway. The existence of such "escape" mechanisms implies that dual targeting of these pathways may lead to superior efficacy and better clinical outcome in selected patients. Several clinical trials targeting one or both pathways are already underway in BC patients. The toxicity profile of this novel approach of dual pathway inhibition needs to be closely monitored.
- Malignant gliomas metastasize throughout the brain by infiltrative cell migration into peritumoral areas. Invading cells undergo profound changes in cell shape and volume as they navigate extracellular spaces along blood vessels and white matter tracts. Volume changes are aided by the concerted release of osmotically active ions, most notably K(+) and Cl(-). Their efflux through ion channels along with obligated water causes rapid cell shrinkage. Suitable ionic gradients must be established and maintained through the activity of ion transport systems.(Haas BR et al 2010)

  EXPERIMENT- The Sodium-Potassium-Chloride Cotransporter Isoform-1 (NKCC1) provides the major pathway for Cl(-) accumulation in glioma cells. NKCC1 localizes to the leading edge of invading processes, and pharmacologic inhibition using the loop diuretic bumetanide inhibits in vitro Transwell migration by 25% to 50%. Short hairpin RNA knockdowns of NKCC1 yielded a similar inhibition and a loss of bumetanide-sensitive cell volume regulation. A loss of NKCC1 function did not affect cell motility in two-dimensional assays lacking spatial constraints but manifested only when cells had to undergo volume changes during migration. Intracranial implantation of human gliomas into severe combined immunodeficient mice showed a marked reduction in cell invasion when NKCC1 function was disrupted genetically or by twice daily injection of the Food and Drug Administration-approved NKCC1 inhibitor Bumex.

  These data support the consideration of Bumex as adjuvant therapy for patients with high-grade gliomas.

  Tamoxifen an antiestrogen frequently used in the treatment of breast cancer and is currently being assessed as a prophylactic for those at high risk of developing tumors. It is shown (Jacob TJ, Hardy SP et al 1994) that tamoxifen and its derivatives are high-affinity blockers of specific chloride channels. This blockade appears to be independent of the interaction of tamoxifen with the estrogen receptor and therefore reflects an alternative cellular target.

Chloride channels as targets for apoptotic cell death in cancer cells

Chloride channels are known to have an important role in a wide variety of cellular functions including cell proliferation, membrane potential, fluid secretions and cell volume regulation. Additionally, chloride channels are known to play a significant role during apoptosis however, they have received considerably less attention than their potassium counterpart. Chloride channel activity has been observed in large variety of cell types undergoing apoptosis either via the extrinsic or intrinsic pathway. Similar to other ion channels, inhibition of chloride channels to prevent apoptosis has been cell type and/or stimulus specific. Early studies reported the importance of chloride channels during apoptosis where the death receptor pathway activated a tyrosine kinase-dependent chloride channel whose inhibition resulted in decreasing cell death. Interestingly, impaired chloride efflux induced by a variety of cell death stimuli was shown to prevent internucleosomsal DNA fragmentation, but not other classical characteristics of programmed cell death. Calcium-activated chloride channels (CaCCs) have been reported to be pro-apoptotic and suppress tumour formation in epithelial cells. However, other family members of the CaCCs along with the recently characterized 8-transmembrane receptor-activated CaCC (TMEM16A) can promote cellular proliferation and tumorigenesis. These studies suggest a variable role of chloride and/or anionic current during apoptosis and cancer progression.

The future pathways

Cancer treatment that involves the targeting of ion channels is known as targeted therapy. Targeted therapy is considerably changing the treatment and prognosis of cancer. Progressive understanding of the molecular mechanisms that regulate the establishment and progression of different tumors is leading to ever more specific and efficacious pharmacological approaches. In this picture, ion channels represent an unexpected, but very promising. The expression and activity of different channel types mark and regulate specific stages of cancer progression. Their contribution to the neoplastic phenotype ranges from control of cell proliferation and apoptosis, to regulation of invasiveness and metastatic spread. The future of cancer treatment using ion channels lies in the improvement of oncochannelopathy and targeting these chloride ion channels. By reducing their over expression, as we have observed, their expression is increased in tumors.

Ion channels are ideal drug targets as the small molecules may be effective from the extra cellular space and do not need to enter the target cells. It is indeed becoming increasingly clear that the inhibition of ion channels is effective in stopping tumour growth and metastasis. The use of channel inhibitors is, however, limited by side effects, because of the fact that chloride channels are also present in the non cancerous cells of human beings. So when a drug molecule acts by inhibition of the channels in large amounts it will also result in inhibition of the channels in non cancerous cells and result in cytotoxicity. Nevertheless, several ion channel modulators are already in clinical trials. Moreover, ion channels are considered as targets for vaccination against tumour-associated antigens. The extracellular domains of the channels and transporters are accessible for antibodies. Several clinical trials and mouse models highlight the feasibility of attacking tumours by targeting channels. Those include the Cl^- channel inhibitor tamoxifen and chlorotoxin or TM-601, substances accomplishing internalization of ClC-3 thus inhibiting migration of glioma cells, which has already been discussed in previous sections.

We have observed that CLC's plays an important role in maintaining pH of the cells by H+ transport. The metabolism of cancer cells differs substantially from normal cells, including ion transport. Although this phenomenon has been long recognized, ion transporters have not been viewed as suitable therapeutic targets. However, the acidic pH values present in tumours which are beyond normal limits are now becoming recognized as an important therapeutic target. Carbonic anhydrase IX (CAIX) is fundamental to tumour pH regulation. CAIX is commonly expressed in cancer, but lowly expressed in normal tissues and that presents an attractive target (Oosterwijk E, Gillies RJ 2013). Thus it can be concluded that proton transport inhibitors can be used as potentially anti cancer drugs. (Harguindey S, Arranz JL, Wahl ML, Orive G, Reshkin SJ. 2009).

For antagonizing the cytotoxic effect a molecule or API can be designed in such a way that it inhibits the over expressed chloride channels in the cells when they will be present at higher levels than a standard concentration and the standard concentration we need to fix. The major drawback of this approach is that some ion channel blockers produce serious side effects, such as cardiac arrhythmias. Therefore, drug developing efforts aimed at producing less harmful compounds are needed and possible approaches toward this goal will be discussed. (Arcangeli, A, Crociani, O, Lastraioli, E, Masi, A. et al 2009)

Conclusion

Chloride ion channels have always been considered extremely valid pharmacological targets. Since they are involved in crucial physiologica functioning of the cells their malfunctioning results in different types of diseases as discussed. Understanding of the involvement of the chloride ion channel family proteins in cancer and tumorigenesis is still in a development state. As evidence suggests that the role of ion channels in cance development is increasing day by day, so development of chloride ion channel blockers to reduce their expression could be used as a potential therapy But the development of the channel blockers is still in development process and research is going on. The major problem of targeting chloride ion channels as their therapeutic targets for cancer is that chloride ion channels are also expressed and present in cytoplasm and cell organelles of the norma cells of our body. So when we target this ion channel they will reduce their expression in non malignant cells and hence will result in seriou physiological problems. As the important roles of chloride ion channels in cell physiology have already been discussed,by targeting this membrane it i inevitable to cause relevant side effects in healthy bodies.

On the other aspect Anoctamin shows impressive effects on basic cell properties, and support both cell proliferation and regulated cell death. Clearly more work is required to be able to define the cellular functions of anoctamins, and their role in proliferation and cancer development. Blocking ANO1 appears feasible to interfere with cancer growth. In contrast to the pro-proliferative effect of ANO1, ANO6 seem to contribute to different types of regulated cell death. Activation of ANO6 may cause swelling or shrinkage of cells, and causes increase in intracellular Ca^{2+}, phospholipid scrambling. It may all contribute to ANO6-induced cell death. Thus, direct activation of ANO6 may be a promising new strategy to induce cell death in cancer cells.

Functional analysis of Ano1 and the other members of the anoctamin family has just begun. These proteins were recognized initially as cancer-associated proteins and are now discussed in the context of ion conductance, volume regulation and phospholipid scrambling. Because ion movement, RVD, lipid scrambling and migration are related and are dysfunctional in cancer and metastasis, anoctamins also have great potential as therapeutic drugs not only against cancer but also other disorders. Their potential role as therapeutic agents has not been yet discovered fully, but it is under research and development. It will be exciting to analyze how inhibitors of anoctamins affect cancer progression, metastasis and the prognosis.

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