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ION CHANNEL MODULATORS AS ANTI-ANGIOGENIC TOOLS IN TUMOR ANGIOGENESIS

Chandana Kamili [*A], Hima Sowmya Kandoti [A], Abbulu Konde [A], Rama Mohan reddy Tummaluru [A], Ravi Shankar Kakaraparthi [B], Uma Maheshwararao Vattikutti [C]

[*a] CMR College of Pharmacy, Hyderabad- 501401 (Telangana), India.
[b] Department of Pharmacology, Sri Sai Aditya Institute of Pharmaceutical Sciences and Research, Surampalem. (Andra pradesh),India.
[c] Department of Pharmacognosy, TRR College of Pharmacy, Hyderabad- 500079 (Telangana), India.

*Corresponding author E-mail: **chandanakamili@gmail.com**

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ABSTRACT

Tumor angiogenesis is the growth of new blood vessels that tumors need to grow. This process is caused by the release of chemicals by the tumor and by host cells near the tumor. Ion channels and aquaporins play a major role in tumor angiogenesis by turning on the angiogenic switch in several types of cancer cells. They induce tumor angiogenesis by promoting cancer development stages like cell invasion, cell migration, cell differentiation, cell proliferation etc. In this review, an overview of the mechanism of angiogenesis and the role of ion channels and their modulators in tumor angiogenesis of different types of cancers are presented. Studies on ion channel modulators will advance our understanding of the molecular genesis of tumor angiogenesis and may identify novel and effective targets for the clinical applications of different types of cancers.

INTRODUCTION

1.1 Ion Channels

Ion channels can be defined as the cell membrane channels such as sodium, potassium, calcium channels, etc., and are specifically permeable to certain ions. Whereas, aquaporins(AQP's), which are also called as water channels are the pore-forming membrane proteins that specifically allow the passage of water while restricting the entry of ios and other molecules. Ion channels and aquaporins also help in important cell processes, for instance, cell volume regulation, programmed cell death, and multiplication of cells.[1]-[5]

1.2 Angiogenesis

Angiogenesis is a fundamental process in living organisms, where fresh blood vessels are produced from the already existing ones[6],[7],[8]. It can be defined as the process of anew production of vascular endothelial cells[9]. It plays a major role in the tissue build up and tissue repair. Nonetheless, it also helps in the

development of tumors. Hence, anti-angiogenic agents can have a good scope from cancer outlook.

2.PROMOTERS OF ANGIOGENESIS : The various promoters of angiogenesis and their functions are described in Table-1.

3.1 Angiogenic Switch: Tumor angiogenesis plays a crucial role in cancer cell growth and development. The steps in tumor angiogenesis are similar to that of physiological angiogenesis[28],[29],[30]. Ischemia and hypoxia are said to be the major initiators of angiogenesis("on" switches).

3.2 Ion Channels: role in endothelial cell growth and tumor angiogenesis: Alteration of different ion channels leads to initiation of angiogenic processes in cancer cells like carcinoma, melanoma and neuroblastoma cells.

4.1 **Calcium(Ca^{2+}) channels:** Ligand-gated and voltage-gated Ca^{+2} channels play a vital role in angiogenesis. Transient Receptor Potential(TRP's) and Inositol triphosphate receptor- Endoplasmic reticulum(IP3R's- ER) release channels which are present in endothelial cells(EC's) also add to the angiogenic process.

Secondary messengers like intracellular Ca^{+2} ions show significant actions in basic cell functions like regulation of cell cycle, cell death, autophagy, gene expression, and cell motility[31].

4.1.1 IP3Rs-ER Ca^{2+} release channels: IP3R-mediated ER Ca^{2+} release involves in the overall intracellular Ca^{2+}signalling network and controls basic cellular functions like cell proliferation and differentiation[32].

4.1.2 Plasma membrane Ca^{2+} channels

4.1.2.1 Voltage-gated Ca^{2+} channels: Voltage-gated calcium channels(VGCCs), which are also called as Ca_v family are formed as a complex of few diverse sub-units such as α_1, α_2, β_{1-4} and γ. They regulate the entry of Ca^{+2} ions with the help of action potential. The Ca_v1 type of VGCC's regulates L-type Ca^{+2} ion influx resulting in muscle constriction and endocrine secretion; the Ca_v2 type regulates N-, P/Q-, and R-type Ca^{+2} influx where they initiate faster neurotransmission while the Ca_v3 T-type Ca^{+2} influx, which leads to an increasingly faster activation and inactivation by action potential[33]. Microarray studies showed VGCC's mRNA gene expressions in various types of malignancies[34]. L-type Ca^{+2} channels are said to play a significant role in the transition of tumors, for instance, colon and esophageal cancers. Whereas T-type Ca^{2+} channels found in breast, ovarian, prostate, colon and esophageal and glioma cells. For instance, $Ca_v3.1a$ acts on the normal adult brain while $Ca_v3.1b$ mostly acts on fetus[35]-[39].

4.1.2.2 TRP channels: The superfamily of TRP channels comprises of in excess of 30 members, which can be additionally divided into 7 subgroups, i.e. TRPA (ankyrin), TRPC (canonical), TRPM (melastatin), TRPML (mucolipin), TRPP (polycystin), TRPN (no mechanoreceptor potential C), and TRPV (vanilloid)[40]. Mammalian TRP proteins form homo or heterotetrameric as non-specific Ca^{2+}-permeable cation channels, which can be activated and controlled by a wide variety of stimuli, such as Ca^{2+}, temperature, pH, Reactive oxygen species[ROS], chemical and mechanical stress. Hence TRP channels are the perfect cellular sensors which play an effective role in tumor angiogenesis and cancer progression[41].

4.2.Potassium(K^+) channels

4.2.1 Calcium-activated Potassium(K_{Ca}) channels: K_{Ca} channels help in the production of vasodilating factors and gene expression by mediating hyperpolarization in EC's. Patients with colonic adenocarcinomas(colon cancer) were taken as subjects and the mesenteric arteries were investigated. This study

proved that a lot of endothelial cells expressed K_{Ca} channels[42]. A 2.7 times increase in hyperpolarization of EC's induced by bradykinin is shown in cancer patients compared to controls due to expanded K_{Ca} channel expression. Hence, K_{Ca} channels have a significant role in tumor angiogenesis. Also, K_{Ca} channels are highly expressed in metastatic brain tumor tissue compared to a typical brain tissue[43]. Bradykinin and other K_{Ca} channel agonists involved in making the blood-brain barrier(BBB) more penetrable in brain tumors and capillary epithelial cells leaky in normal brain tissue. Large conductance Ca^{+2}-activated K^+ (BK_{Ca}) channels, when blocked therapeutically result in the inhibition of proliferation induced by FGF-2 in human umbilical vein endothelial cells(HUVEC's). FGF-2 has the ability to increase the action of BK_{Ca} channels, thus enhances BK_{Ca} channel activity in the formation of new blood vessels[44]. BK_{Ca} channels also showed strong activity in cell proliferation induced by VEGF-A and FGF-2. It was investigated that the selective blockade of BK_{Ca} channels did not have any effect on cell proliferation[45]. Hence, it can be said that the activity of BK_{Ca} channels in pro-angiogenic activities is influenced by some extra factors.

4.2.2 Voltage-gated Potassium channels: Voltage-gated potassium channels are tetramers of 4 similar isomers in a ring. They regulate Ca^{+2} ion concentration along with controlling the production of VEGF by tumor cells. Oncogenic Eag1(K_v10.1, KCNH1) is expressed in xenograft tumors induced in mice by implantation. Due to enhanced VEGF production and release, K^+ channels involved in increasing vascularization which is related to HIF-1[46]. It was studied that Eag 1 does not interfere with the HIF-1 control pathway during hypoxia. Human Erg 1(K_v11.1, HERG, KCNH2), which is selectively inhibited therapeutically is highly expressed in gliomas. This resulted in reducing the release of VEGF by glioma cells[47]. Hence, Erg 1 channel has a significant role in cancer cell growth by increasing neoangiogenesis which is commonly seen in high-grade gliomas.

4.3 Voltage-gated sodium (Na^+) channels: VGSCs comprise of a pore-forming α subunit, commonly crosslinking with at least one identical or different, smaller β subunits[48]. Nine genes in humans (SCN1A to SCN5A, and SCN8A to SCN11A) code for nine distinct VGSC proteins (Na_v1.1 to Na_v1.9, respectively) related to differences in their α subunits [Table-2][49]. Voltage-gated sodium (Na^+) channels (VGSCs) showed expression in both excitable and non-excitable cells[50]. They influence cell motility, cell proliferation, secretion, differentiation, and phagocytosis[51]-[54]. Thus, they play a vital role in both physiological and tumor angiogenesis. Hence, VGSC's are studied further for their therapeutic activity[55]. Nav1.5 controls cell invasion in colon cancer[56], whereas Nav1.6 enhanced tumor cell invasion in non-small cell lung cancer and cervical cancer[58]. Similarly, Nav1.7 improves cell invasion in different types of cancers[57]. Studies conducted on MAD-MB-231 breast cancer cell line revealed that the NAV1.5 Na^+ channel promoted tumor cell invasion by enhancing Na^+ influx and action potential in the cells[59],[60],[61]. Hence, It can be said that VGSC's play a major role in tumor angiogenesis.

4.4 Aquaporins(AQPs): Aquaporins are a part of a special superfamily of membrane integral proteins known as intrinsic proteins. Aquaporin proteins are comprised of a bundle of six transmembrane α-helices. They mainly help in the regulation of cell volume and body water homeostasis, hence play a major role in cancer cell growth[62],[63]. AQP0 to AQP12 of this family were reported. They were divided into three

subgroups based on their primary sequences: water selective (AQP0, 1, 2, 4, 5, 6, and 8), aquaglyceroporins (AQP3, 7, 9, and 10), and superaquaporins (AQP11 and 12)[64].AQPs have been appeared to be vital for cancer cell growth. For instance, AQP3 involves in the activation of ERK1/2 which increases the expression of MMP-3 and secretion. Thus it regulates cell invasion and motility in prostate cancer[65]-[68][Table-2].

Ion channel modulators: An ion channel modulator is defined as a drug/an agent that acts by altering the function of ion channels. They may be channel blockers or channel activators[69].

Ion Channels as drug targets in cancer: Ion channels highly influence the genes involved in malignant transformation and this results in the activation of selective cellular responses. Hence, the pathophysiology of cancer can be affected by the alterations of genes expressing different ion channels. Also, ion channels act from outside the target cells. All things considered, ion channels can be used as perfect therapeutic targets in malignancy[70].

Ca^{+2} channel modulators: As Ca^{+2} channels help in the activation and inactivation of various steps in tumor angiogenesis mechanism, they can be considered as potential targets for the treatment of malignancy.

6.1.1 Voltage-gated Calcium channel (VGCC's) inhibitors: VGCC blockers are the agents affecting Ca^{+2} signaling and are the first investigated ion channel modulators. VGCC blockers find use in cardiovascular and central nervous system disorders[71]. Many investigators have collected proofs that VGCC's play significant roles in various types of cancers. This led the investigators to launch investigations to reuse the FDA approved VGCC blocker drugs for treating malignancy conditions [52]. L-type VGCC blockers of different structures were tested for their potent blocking action on breast cancer progression[72]. Amlodipine, which is a dihydropyridine Ca^{+2} channel blocker was investigated and it was found that amlodipine blocked the progression of human epidermoid carcinoma A431 cells. This action was shown both in vitro and in vivo by arresting cell cycle at G_1 phase[73]. Similarly, mibefradil, an anti-hypertensive drug is a T- and L-type Ca^{+2} channel blocker. Even though, it was withdrawn from the market due to its adverse effects on metabolizing enzymes of the body, sit showed anticancer effect by effectively reducing the tumor size. This was proved through the investigations done on glioma animal model and pancreas xenograft animal model[52],[74]. Also, NNC-55-0396 is a new mibefradil derivative has [potent Ca^{+2} channel targeting capacity with less adverse effects on metabolizing enzymes[75]. Hence, this compound can be used as an anti-angiogenic agent which efficiently inhibits tumor angiogenesis[52],[77]. Flunarizine, a T-type Ca^{+2}, and sodium channel blocker have significant anti-angiogenic action by inhibiting cell proliferation, migration and tube formation[76][Table-2].

6.1.2 Potassium (K^+) channel modulators: Voltage-gated K^+ channels (Kv), calcium-activated K^+ channels (K_{Ca}) and ATP-sensitive K^+channels (K_{ATP}) are the various K^+ channels that are involved in the progression of tumor angiogenesis[78],[79]. K_v10.1 (EAG), K_v11.1 which is a human ether-a-go-go-related gene(hERG) and K_{Ca}3.1 are the genes whose therapeutic targeting is used in the treatment of cancer. Investigations were done to design a monoclonal antibody that functions selectively against K_v10.1. It blocked the action of the channel which resulted in decreased growth of colonies in cancer cell lines. It also inhibits the tumor growth of MBA-MB-4355 cancer cell lines *in vivo* [80].

S.no	Promoter	Function	References
1.	Vascular Endothelial Growth factor (VEGF)	-Production of nutrients for tumor growth	[10]-[13]
2.	Angiopoietins(Ang)	- Help in the formation of mature blood vessels	[14]-[17]
3.	Matrix metalloproteinase(MMP)	-Causes proteolysis and helps in sprouting angiogenesis	[18]-[21]
4.	Delta-like ligand 4(DLL-4)	-Negative regulatory effect on angiogenesis	[22],[23]
5.	Class-3 Semaphorins(SEMA3s)	-Regulate angiogenesis by modulating EC adhesion, migration, proliferation, survival and recruitment of pericytes	[24]-[27]

Table 1: Promoters of Angiogenesis

Table 2: **Role of ion channels and their modulators in different types of cancers**

S.no	Ion channels	Ion channel subtype	Gene encoding the ion channel	Function in oncogenesis	Disease	Ion channel modulator	Mechanism of ion channel inhibitor	References
1	Calcium channels	a)Voltage- gated Calcium channels Cav1.1-1.4 Cav2.1-2.3 Cav3.1-3.3	-Cacnal s,c,d,f -Cacnal a,b,e -Cacnal g,h,i	-Proliferation -Tumor angiogenesis	-Adrenal -Adenomas -Prostate cancer -Melanoma -Glioblastoma -Gastric cancer -Colon and Esophageal cancer	**Small molecule inhibitors** -Cav -Mibefradil -TTL1177 -Endostatin siRNA/shRNA -Cav3.1 -Cav1.3 -Flunarizine -Nifedipine **Activators** -HC-030031	-Reduced cell proliferation by inducing apoptosis -Reduced cell proliferation, migration, tube formation -Reduced proliferation and related angiogenesis -Reduced cell proliferation and metastasis	[31],[92]
		T-Type						
		L-Type						
		b)TRP channels TRPA	Trpa1	-Cell migration and metastasis Nociception,Inflammation	-Breast cancer -Esophageal carcinoma -Gastric cancer			

		TRPC TRPM TRPV c)IP3R's IP3R1 IP3R3	Trpc1-7 Trpm1-8 Trpv1-6 - -	-Tumor angiogenesis -Tumor angiogenesis -Nociception -Cell proliferation -Tumor angiogenesis -Cell migration -Tumor angiogenesis	-Colo-rectal cancer -Melanoma -Bladder and Prostate cancer -Glioma, Lymphocytic Leukaemia -Glioma, Gastric, Colon, Head and Neck Cancer	<u>-Englerin A</u> **<u>Inhibitors</u>** -Xestospongin B, -Xestospongin C **<u>Inhibitors</u>** -Heparin, Caffeine	-Reduced proliferation -Reduced cell migration	[57],[58] [47],[48] [49],[92]
2	Potassium channel	a)Voltage-gated Potassium channels Kv1.1-1.8 EAG 1,2 hERG 1-3 or Kv11.1-11.3 b)Calcium activated Potassium channels -Kca 1.1 orBKca -Kca2.1-3 or SK1-3	Kcna1-7,10 Kcnh 1,5 Kcnh2,6,7 Kcnma 1 Kcnn1-3	-Proliferation of cancer cells -Apoptosis resistance -Immuno-suppresive action -Migration of breast cancer cells -Tumor angiogenesis Proliferation of cancer cells -Apoptosis	-Cervical cancer -Breast cancer -Ovarian cancer -Osteo-sarcoma -Colorectal cancer -Glioma -Melanoma -Small cell Lung cancer -Ovarian cancer -Prostate cancer -Breast cancer	**<u>Small molecule channel inhibitors</u>** -hERG1 K+channel -Kv10.1; Astemizole, Imipramine -Kv11.1; Way123398, E4031 **<u>Small molecule channel activators</u>** -NS1643 Antibody -Kv10.1 **<u>Toxins</u>** -Kv11.1; Ergotoxine siRNA/shRNA -Kv10.1, Kv11.1 -4-aminopyridine **<u>Small molecule channel activators</u>**	-Reduced proliferation by increasing apoptosis or cell cycle arrest at G0/G1, G1/S or G2/M phase -Prevents angiogenic switch -Reduced metastasis -Reduced progression of the cell cycle -Reduced proliferation	[63],[64],[92]

		-Kca3.1 or IKca or SK4 -KATP Channel	Kcnn4	-Cell migration and metastasis Tumor angiogenesis		-NS1643		[58],[59]
3	Sodium channel	Voltage-gated Sodium channel Nav1.5 Nav1.6 Nav1.7 Nav1.9	Kcnma1	-Proliferation	-Prostate cancer -Ovarian cancer -Colon cancer -Cervical cancer -Non-small cell lung cancer (NSCLC) -Breast cancer	**Small molecule inhibitors** -Phenytoin, -hydroxyl amide -Ranolazine -Riluzole **Toxins** -Tetradotoxin	-Reduced cell proliferation via cell cycle arrest -Anti-metastasis	[56],[57],[58]
4	Chloride channels	ClC2	-	-Proliferation -Regulation of cell volume -Cell migration		**Agonists** -Lubipristone **Inhibitors** -Mefloquine	-Negative regulation of cell volume -Reduced cell migration and proliferation -Reduced cell migration and proliferation	[93]
5	Aquaporins	AQP-1 AQP-3 AQP-4 AQP-5 AQP-8	Aqp 1 Aqp-3 Aqp-4 Aqp-5 Aqp-8	-Angiogenesis -Proliferation -Migration -Malignancy	-Gastric cancer -Prostate cancer -Glioma -Breast cancer -Cervical cancer	miR-874	-Reduced proliferation	[64]-[67]

Astemizole, a potent hERG potassium channel blocker, reduced MB435S xenograft tumor growth by approximately 30% and growth of EAG-expressing CHO by 50%[77]. Astemizole and calcitriol are used in combination therapy in mice with T-47D (human breast cancer cell line). The result showed 85% inhibition of tumor volume. Hence, combination therapy is preferred than either drug alone[81]. 4-Aminopyridine, a K^+channel blocker inhibits proliferation and related angiogenesis by blocking voltage-gated K^+ channels which play a vital role in the progression of cell cycle[82]. [Table-2]

6.1.3 Sodium(Na^+) channel modulators

$Na_v1.5$, $Na_v1.6$ and $Na_v1.7$ and their subtypes are highly expressed in many types of malignancies like breast, prostate, lung, leukemia and cervical cancer[80]. In breast cancer, n$Na_v1.5$, which is a neonatal isoform of $Na_v1.5$ regulates the high influx of Na^+ ions into the cell, resulting in calcium homeostasis[83]. VGSC's enhance cell invasion by stimulating cysteine-cathepsin activity in breast, prostate, and lung cancer cells[84]. Many investigators conducted preclinical studies to describe the therapeutic targeting of VGSC's. The results showed a reduction in tumor growth and metastasis formation. α-hydroxy-α-phenylamides are the new class of compounds, which have a strong inhibitory action on VGSC's. An enantiomer of 3-chlorophenyl-α-hydroxyamide is synthesized and administered having prostate cancer cell line PC3 xenografts. The result showed a 60% decrease in tumor volume[85]. Significantly, hERG showed up to 16% decrease in tumor volume. Studies conducted on RS100642, a VGSC blocker showed that the administration of the drug resulted in an increase in the survival of by 50 days in 7,12-dimethylbenz(a)anthracene(DMBA)-induced breast cancer in mice[86]. Similarly, tetrodotoxin, which is also a VGSC blocker reduced metastasis and prolonged survival in Mat-LyLu cell-induced prostate cancer model[87]. Phenytoin reduced tumor growth by 30% in breast cancer cell line MDA-MB-231 expressing $Na_v1.5$.

6.1.4 Aquaporins(AQPs): Numerous inhibitors of aquaporins may be useful in treating tumors[88],[89],[90]. AqB013, an AQP1 inhibitor showed anti-angiogenic activity by potentially reducing the progression of various angiogenesis steps in colon cancer[91]. Whereas copper sulfate($CuSo_4$), an AQP3 inhibitor is used in pancreatic cancer[92]. Similarly, small molecule AQP modulators and pharmaceutical formulations like WO2013005170 (metal-based inhibitor of aquaglyceroporins AQP3, AQP7, and AQP9), US8835491 (modulator of orthodox AQP1), and WO2008052190 (modulators of orthodox AQP4) find use in clinical application [88][Table-2].

7. CONCLUSION

Angiogenesis, the formation of new blood vessels is a normal process during the growth of the body and in the body's replacement of damaged tissue. However, it can also occur under abnormal conditions, such as in tumor progression. At some point, after months or even years as a harmless cluster of cells, tumors may suddenly begin to generate blood vessels apparently because they develop the ability to synthesize certain growth factors that stimulate the formation of vessels. Many ion channels and AQPs are differentially expressed in tumor tissues and cells. Changes in the expression or activity of ion channels or AQPs give the tumor cells a pathological character and the ion channels then affect various aspects of the malignant behavior of tumor cells, such as proliferation, migration, and invasion. Multiple signaling pathways may be activated by ion channels and AQPs and this could enhance a variety of oncogenes, thereby contributing tumorigenesis and progression. Inhibiting the expression or blocking the activity of ion channels or AQPs impairs tumor cell function both in

vitro and in vivo, which could open a new avenue for pharmaceutical research in cancers. As for neoangiogenesis, ion channels are involved at all stages, in tumor cells, as well as endothelial cells or stromal cells. Ion channels thus represent a new and promising field of research concerning the development of novel therapeutic agents.

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