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Role of PKCα in Bladder Cancer Progression

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Abstract: Bladder cancer is the most common form of epithelial cancer. Treatment regime generally based on endoscopic removal of tumor with or without immunotherapy. Treated cause frequently recurr with more profound invasion characterized by metastasis that is most severe form of disease associated with number of death worldwide. During the curse of invasion, cancer cell remove from tumor by dissolution of tight junction and migrate through nearby blood vessel by dissolution of endothelial cell tight junction. In this way cancer cells are metastasize anywhere else. Ultra structure study revealed that four basic transmembrane protein namely claudin, occludin, JAM and tricellulin are forming structure of tight junction sealing strand. Claudin is critical transmembrane protein of tight junction. Their activity is in the state of flux which ultimately determine the functioning of tight junction. Phosphorylation seems to play an important role in regulating the activity of claudin. Depending upon the tissue, it's activity may enhance or diminish by phosphorylation. PKC is one of the phosphorylating kinase evolutionary chosen by cell for this purpose. PKC have different isoforms Artificial stimulation of PKC by phorbol ester established its role as a tumor promoter i e., as a oncogene. Once it has been activated to membrane they phosphorelate various protein depending on the cell type. Since a given cell have characteristics range of protein that never be found in other cell, a given PKC have different range of phosphorylating target in different cell. Indeed a given PKC in different stage of cell may found different protein target. Based on the fact that the C - terminal cytoplasmic domain of claudin posses ser/thr phosphorylation site and upregulation of PKC alpha in bladder cancer, it seems likely that the PKC alpha might be a phosphorylating kinase for claudin deregulation for this purpose.
Keywords- PKCα; Bladder Cancer; claudin.

1. INTRODUCTION:

Bladder cancer is the most common cancer of the urinary tract develops from the unrestrained proliferation of cell lining the bladder. Epidemiological data rank it the ninth most common cancer in the world (1). Men are generally four times more prone to develop bladder cancer compared to woman probably because of the occupational structure that increase the risk of carcinogen exposure (2) .The risk factor include tobacco smoking, exposure to heavy metal such as Arsenic, old age and recurrent UTI (3).Transitional cell carcinoma is most common form of bladder cancer account for 90% of the reported causes . Apart from this major accounting cause of bladder cancer, squamous cell carcinoma develops from squmous cell and adenocarcinoma develops from glandular cell is less commonly reported (4). Sarcoma from bladder is rarest (5). Superficial and non muscles invasive bladder cancer can be treated endoscopically, with or without immunotherapy or adjuvant or neoadjuvant chemotherapy. Recurrence of superficial and non muscles invasive bladder cancer(account in 50 to 70% surgically treated cases) leads to more severer condition characterized by local invasion or metastasis to distant organ that need the removal of bladder by radical cystectomy. Nevertheless, invasion and metastasis reflecting the advance stage henceforth remains a principal cause of death from bladder cancer (6).

Tight Junctions:

The existence of anatomical barrier within the human body virtually relies on the arrangement of epithelial as well as endothelial cell to separate out two non co - existent liquid. For example, capillary endothelial cells provide the basis for blood brain barrier that protect the brain from harmful substances circulating in the blood (7) .This feat is accomplished by forming a sheet of cell by connecting two adjacent cells through a sealing strand (8). These barrier are designed in such a way to be selective for the movement of one type of molecule while restrict the movement of others (9). Consequently two different cellular pathway exist for movement of molecule : one is transcellular pathway in which selective molecule travel through the cell by endocytosis and exocytosis to cross the barrier and the other one is paracellular pathway in which molecules move by diffusion or osmosis between the two adjacently connected cells . Tight junction is an apex structure of junctional complex play an important role in construction of barrier permeability particularly through paracellular pathway (10). The number of tight junction as well as their composition which vary from tissue to tissue considerably responsible for determining the permeability of barrier which is generally assayed by transepithelial resistance (11). Membranes with reduced number of tight junction have higher transepithelial resisistance (12). Conversely, lower transepithelial resistance has been

associated with higher number of tight junction. This is exemplified by higher number of tight junction and least permeability of blood – urine barrier (13). In fact, it is the highest known impermeable barrier in human body (14).

Tight junctions also provide a fence for cellular compartmentalization of protein and lipid. So as an intact location of these component remain inside the plasma membrane for various cellular task , such as to maintain polarity (10). Apart from these two important function, tight junction are also required for maintaining the tissue architecture because of the cell to cell adhesion, that often loss in various disease including cancer (15).

Cancer progression described as the dislodgement of cells from primary tumor and their movement through adjacent blood or lymphatic vessel to establish anywhere else is characterized by metastasis. It is associated with disruption of tight junctions both from the tumor tissue to stimulate invasion and from the nearby endothelial cell to metastasize to distant organ (16). Loss of tight junction also provide a leak within the tumor microenvironment by secreting various growth factor and associated protease that rocketed the tumor growth further (10).The disruption of delicate proportion between oxidative stress and antioxidant level in body also influence tight junction integrity (17). This is implicated in the disruption of tight junction (18).

Organization of Tight Junction:

The tight junction is organized by four classes of transmembrane protein namely occludin, claudin, JAM and tricellulin (19).The first identified transmembrane protein ocludin have a single polypeptide of 65 kd with four transmembrane domain, two extracellular loop and three cytoplasmic domain(20). Occludin knockout mice still able to form intact TJ (Tight Junction) strands suggesting a minor role of occludin in morphology of tight junction (21). Claudin, the second identified Tj proteins also have four transmembrane domains and two extracellular loops just like occludin (22). Inspite of similar organization, amino acid sequence differs substantially between ocludin and claudin. To date 24 types of claudin have been identified in human with a characteristics tissue specific distribution (23).

One another class of integral membrane protein which belongs to Ig super family is a JAM which has a single transmembrane domain and single cytoplasmic loop with two extracellular domains(24). It has been found apart from epithelial and endothelial cell to leukocyte and platelet suggesting to there more common role apart from maintaining tight junction integrity. Tricellulin which is generally downregulated during epithelial to mesenchymal transition, is a single polypeptide with four transmembrane domain found mainly at tricellular junction having some sequence similarity with occludin (25). Angulins was recent found single transmembrane protein required for localization of tricellulin at tricellular contacts (26).

All transmembrane protein stabilize by their interaction with a verity of adaptor protein such as ZO1,ZO2,ZO3,PAR3,Afadin, and cingulin which generally have conserved ~80 amino acid PDZ domain for this purpose (27). Except claudin 12 , all caludins have carboxyl terminal PDZ domain for their interaction with other protein such as ZO – 1 , ZO – 2 etc .This interaction facilitate strand organization. Cytoskeleton protein including actin, myosin, and microtubules play an important role in the organization of tight junction. This is exemplified by localization of actin polymerizing protein namely Arp2/3, N- WASP, cortactin and VASP. ZO protein itself binds directly to actin. Non muscle derived myosin are found to be localize in tight junction. Further study demonstrates that microtubule linked with phosphorylated cingulin (26) .Various regulatory proteins such as Rho GTPase also regulate assembly of sealing strand by their interaction both from transmembrane and adaptor protein. (28).

Claudins in Cancer:

Post translational modification seems to play an important role in regulation of claudins. These include S-acetylation, palmitoylation and phosphorylation. Among this phosphorylation play a critical role in the regulation of claudins. This is so since that C – terminal cytoplasmic domain of claudins posse's serine/threonine phosphorylation site. The consequence of phosphorylation is either increase or decrease tight junction function (29).For example, claudin - 3 phosphorylation by Protein kinase A decreased its activity in ovarian cancer cells while PKC – theta mediated phosphorylation is a prerequisite for the assembly of claudin – 1 and claudin – 3 in intestinal epirhelial tight junction (30). Claudin – 11 level was significantly reduced in T24 cell line. Its forced expression substantialy impedes the invasion while increasing cell matrix adhesion and growth rate (31).This suggest that the restoring tight junction integrity could reduce the migration of disease. Moreover, claudin – 4, and claudin – 7 has been reported to be down regulated (32-33).

Protein Kinase C:

Protein kinase C (PKC) is a peripheral membrane protein belongs to the class of serine/ thronine kinase. The inactive form is found in cytosol fraction of a cell (34). Structurally it is a single polypeptide having one catalytic domain and one regulatory domain. Regulatory domain binds to Diacylglycerol (DAG) or other second messengers depend upon isoenzyme type. Catalytic domain becomes a part of active site and binds to substrate being phosphorylated (35). The amino terminus end have a sequence - A-R-K- G-A-L-R- Q-K-. This spectacular sequence is critical because the consensus sequence for PKC is X-R-X-X-(S,T)-Hyd-R-X in which Hyd refers to a large, hydrophobic residue. This sequence from PKC "pseudo substrate sequence" is very critical because it resembles the substrate sequence except that it has an alanine residue in place of the serine or threonine residue; so it cannot

be phosphorylated (36). PKC also have a property of autophosphorylation. It able to remove phosphate group from ATP in a substrate and cofactor dependent manner (35). The regulatory domain of PKC consists of zinc finger motif containing six cysteine residues with two zinc atom. Because of the presence of cysteine they are susceptible to oxidation by free radicals that damage zinc finger confirmation. This enables the PKC to be catalytically active irrespective of calcium or phospholipids present in cell. In some PKC isoform, oxidative stress triggered translocation from cytosol to plasma membrane while other translocates to nucleus (37). PKC translocation can be revealed by their fusion with green fluorescent protein (38). Immunohistochemistry are also used to localize PKC(39).

Molecular method specially Real – time PCR has revolutionized the study of expression of a PKC gene in a given moment of time (40). In the year 1991, Mochly-Rosen and co-workers identified PKC anchoring proteins and named them 'receptors for activated C kinases (RACKs). Acivated PKC bind RACK (42).

Based on the second messenger requirement, they are grouped into three classes:

Conventional classes - Three isoenzymes namely PKC – α , PKC – β and PKC – ϒ are grouped in this category (Depend on calcium and diacyl glycerol).

Novel classes - Four isoenzymes namely PKC – δ, PKC – ϵ , PKC – η and PKC – θ are grouped into this category (Depend on calcium and diacyl glycerol).

Atypical classes - PKC – ζ and PKC – λ are member of this group (Depend on phosphophatidylserine) (43).

Two classes of receptor namely G protein coupled receptor and tyrosine kinase receptor activate PLC – β and PLC - ϒ respectively (27). Once activated PLC cleaved PI (4, 5) P2 to generate IP3 and diacylglycerol. After formation, IP3 diffuse out to cytosol because of polar nature while diacylglycerol remain bound to membrane due to its non polar nature. Diacylglycerol directly activate PKC while IP3 stimulate the release of calcium ion from endoplasmic reticulum that in turn activates PKC. Both process act simultaneously to generate a cascade of active PKC (36). The activated PKC able to phosphorylate a varity of cellular proteins depending upon cell type. One notable example is the phosphorylation of cell specific transcription factor thereby represses the synthesis of certain mRNA. (24). PKC α , PKC β , PKC ϵ, PKC δ, PKC ζ have been characterized in bladder cancer and in their normal counterpart (44).

Role of PKC in Cancer:

A synthetic compound namely Phorbol esters has been significantly attributed for revealing the role of PKC in cancer. Phorbol ester mimic the role of diacylglycerol but unable to metabolize as the diacylglycerol does. Hence its continous presence activates PKC in aberrant manner that contribute to tumor development (45). PKC show wide reaching effect on cellular environment because of its pleotropic nature (46). In cancer cells PKC activate proliferation associated signaling pathway including mTOR pathway and MAP kinase pathway and inhibit the apoptotic pathway (47).PKC not only activate proliferation but it also associated with its inhibition depend on the state of cell cycle. When Phorbol 12 – myristate 13 acetate (PMA) has been administered in non small cancer lung cell,it block cell cycle progression at different stage of cell cycle depend on the type of isozyme involved. For example G2 to M stage of cell cycle is arrested by PKC alpha. This suggest that PKC play a regulating role in cell cycle by blocking further progression (48).

Role of PKCα in Cancer:

Mammalian PKC - α consist of 672 amino acids and distributed in all the tissue (41). PKC – α , the first identified PKC, has been found to be associated with cancer but their expression varies according to the type of tumor tissue. In bladder and breast cancer it is upregulated reflecting their oncogenic status. In colorectal and malignant renal cell carcinoma it is down regulated (35). Nicotine and tar free cigarette smoke (CSE) extract has been found to induce PKC – α in rat glioma c6 cell (49). H. Pylori induced phosphorylation of PKC – α induce secretion of matrix metalloprotease (50).

Morever, transfection of PKC - α in bladder cancer cell increases resistance against adriamycin.PKC – α activate proliferative pathway Including RAS pathway. GO6976, a PKC – α inhibitor reduce the proliferation of cells by arresting cell at G0/1. Knockdown of PKC - α in bladder cancer T24 and 5637 cell line promote apoptosis (47).

Protecting Proteolytic Degradation of Tight Junction:

Matrix metalloprotease (MMP) , a zinc containing tissue remodelling protease activated by proteolytic cleavage have been implicated in various stage of cancer progression including invasion, angiogenesis and metastasis. Gene knockout study shows that MMP could be a critical target in cancer(51).In normal tissue MMP level is balanced by their specific inhibitor called TIMP (Tissue inhibitor of matrix metalloprotease). A balance between MMP and TIMP is immense importance for normal homeostasis. Disruption of these balance is associated with invasion(52).

MMP 2 (gelatinase – A) and MMP 9 (gelatinase – B) has been found to be associated with invasion and metastasis of bladder cancer cells. (53). Various growth factor including TGF beta induce endothelial MMP expression leading to TJ dysregulation during glioma mediated BBB (Blood brain barrier) impairment (54). Leukemic cell derived MMP 2 and MMP – 9 leads to the degradation of occluding and ZO1 and Claudin 5 of blood brain barrier as a part of their effort to invade CNS (55). A positive correlation established from these finding which support the view that MMP might be directly involved in the proteolytic degradation of TJ.It seems logical that inhibiting the

MMP activity restore the tight junction integrity. Indeed it happens. Various correlative study justify this assumption. For example, broad spectrum MMP inhibitor BB – 1101 increase the claudin - 5 and occludin level as shown by realtime PCR analysis (56). A number of herbal compound show some promising effect to reduce the level of MMP thereby reduce invasion and migration of cancer cells.A positive association has been found between over expression of PKC isoforms and MMP. For example, in breast cancer over expression of PKCα significantly attributed for high level expression of MMP2/MMP9 (47).

2. DISCUSSION:

PKC isoforms has long been recognized as a critical target for cancer. As in many cancers, there unusual expression also affect bladder cancer progression. The PKC isoforms has a multiple target inside the cells and a given PKC isofoms has a specific tissue distribution .It is very unlikey that PKC isoforms itself could be a target of inhibition. This is so since that in normal cells they are also present in a substantial quantity where they phosphorylate target protein. It seems possible that in cancer cells they phosphorylate the same target protein that could behave aberrantly or unique cancerous protein that modulate the progression of cancer. From this perspective an approach can be develop in which PKC target protein could be genetically modified by replacing PKC consensus sequence for a different amino acid of target protein through a site directed mutagenesis approach. The homozygous transgenic mice develop in this way could be useful in study of bladder cancer.

3. CONCLUSION:

Despite of much wealth of biological knowledge about bladder cancer, the treatment remains unsuccessful, eventually causing death of millions of peoples worldwide. Usually, the bladder cancer is originated from the inner most layer of urinary bladder but it can also be originated from other layers. Recurrence leads to more severe condition of disease. Tight junction form seal between two adjacent cell therby creating a barrier that able to separate two different types of liquid in body. The disruption of these barriers is a prerequisite for cancer cell to metastasize. Henceforth, transmembrane protein from which tight junction is constructed is very critical target. Strengthing the tight junction rely on this transmembrane protein, most notably claudin. In bladder cancer there level is either upregulated or downregulated. Phosphorylation plays an important role in regulation of these claudin. PKC, whose expression is aberrant in many cancers has been found to phophorylate claudin in many cancers including bladder cancer. Phosphoryltion either increase or decrease the function of claudin. Each PKC isoforms has specific tissue distribution and specific role. PKCα has been unregulated in bladder cancer while claudin4 level is down regulated. PKCα might be either directly or indirectly through the activation of MMP affect the claudin 4 in bladder cancer.

CONFLICT OF INTEREST:

There is no conflict of interest.

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