Chapter

Implication of Connexin 43 as a Tumor Suppressor in Pathogenesis of Breast Cancer

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Abstract

Breast cancer (BC) is a global public health burden, constituting the highest cancer incidence in women worldwide. Connexins 43 proteins propagate intercellular communication, gap junction intercellular communication (GJIC), remarkably expressed in several tumor types including liver, prostate, and breast. This domain of Cx43 possesses functionally critical sites identical to those involved in gating of channel and phosphorylation sites for various kinases. However, the mechanism by which Cx43 down regulation occurs in breast cancer is far from clear. Several mechanisms like Cx43 promoter hyper-methylation or a cancer-specific reduction of Cx43 expression/trafficking by the modulation of various components of the Cx43 life cycle give the idea to be involved in the down regulation of Connexins in mammary glands, but irreversible mutational alterations have not yet been proved to be among them. Summarily, the efficacy or specificity of these drugs can be increased by a combinatory approach considering an effect on both the Connexins and their regulatory molecules. This chapter will summarize the knowledge about the connexins and gap junction activities in breast cancer highlighting the differential expression and functional dynamics of connexins in the pathogenesis of the disease.

Keywords: Breast Cancer, Connexin, Tumor Suppressor, Gap junction, mammary gland

1. Introduction

Cancers that originate from the breast tissue are called as Breast cancers. Quite often, these cancers originate from the epithelial cells lining the milk ducts or lobules supplying the ducts with milk [1]. Sub-classification of breast cancer into various types is done on the basis of certain characteristics that the cancers develop depending on their origin i.e. whether cancer originates from glandular portion or ductal portion of the breast. Accordingly, cancers that stem from lobules are called as lobular carcinomas, whereas those stemming from ducts are called as ductal carcinomas. Once Primary tumors become invasive, it spreads beyond its place of origin (Breast) to the regional lymph nodes. It may also metastasize i.e., expand to different organ systems of the body, thereby becoming systemic in nature. On the basis of this expansion, breast cancer is of two types Non-invasive or in-situ and invasive. A non-invasive or in-situ cancer is one where the cancer cells

remain confined to boundaries of the lobular unit or draining duct of their origin. On the other hand, cancer cells that traverse outside the basement membrane of the lobules or ducts into the surrounding normal tissues are classified as invasive cancers. Apart from these, there are other types of breast cancer with different stages, varied aggressiveness and different genetic makeup. These factors greatly affect the chances of survival of a patient. Several breast cancers are up regulated by estrogens. These cancer cells carry estrogen receptors on their surfaces and are called Estrogen Receptor-positive cancers or ER-positive cancers. Similarly, some women suffer from another type of breast cancer called as HER2-positive breast cancer. HER2 is a gene responsible for cell growth, division and repair. Increased copy number of HER2 gene may result in faster growth of cancers. Women with HER2-positive breast cancer have higher incidence of disease recurrence making it as a risk factor for breast cancer recurrence. The disease is also more aggressive than women who do not have this type of breast cancer.

2. Stages of breast cancer

Expansion of a cancer determines its stage. Stages of a cancer indicate whether the cancer is limited to the area of origin or has spread to other healthy tissues of the body. Four important characteristics determine a cancer stage:

a. Size of the cancer.

b. Type of cancer i.e. invasive or non-invasive.

c. Has cancer reached lymph nodes,

d. Has cancer metastasized to other body parts.

Firstly, on the basis of extent of the cancer, it can be classified as local, regional or distant. A cancer is **local,** when it is confined within the breast (where it originated). It is **regional** when lymph nodes are involved. And it is **distant** when it has metastasized to other body parts as well.

There is another staging system used to describe the cancer called as TNM staging system. The TNM System is based on three components - size of the tumor (denoted by T), involvement of the lymph node (denoted by N) and whether the cancer has metastasized (denoted by M).

Stage 0: It is a non-invasive stage, during this stage cancer is present at its origin e.g., Ductal Carcinoma In Situ (DCIS). There is no indication of the cancer cells or non-cancerous abnormal cells traveling beyond their origin to neighboring normal tissues.

Stage I: This stage describes an invasive breast cancer i.e., cancer cells invade neighboring normal tissues. There are chances of microscopic invasion in this stage. In such an invasion, the cancer cells have just begun to travel outside the boundaries of their duct or lobule. However, the invading cancer cells are not more than 1 mm.

Stage II: This stage is further sub-categorized into stages IIA and IIB, both describing a different invasive breast cancer. Stage IIA refers to an invasive breast cancer where the tumor cannot be located in the breast but lymph nodes (axillary) under the arm show presence of cancer cells.

Stage IIB: Refers to an invasive breast cancer where a tumor sized between 2 cm and 5 cm has spread to axillary lymph nodes.

Stage III: This stage is further sub-categorized into three stages - Stages IIIA, IIIB and IIIC. Stage IIIA refers to an invasive breast cancer where either the tumor cannot be located in the breast but cancer is found in axillary lymph nodes which are clumped or clinged to other structures, or lymph nodes at breast bone may be involved too.

Stage IIIB: Defines an invasive breast cancer stage where cancer has involved chest wall or breast skin or both and may involve axillary lymph nodes and showing Stage IIIA like features too.

Stage IIIC: Refers to an invasive breast cancer where there is no evidence of cancer in the breast or, in the event there exists a tumor, it is of any size, which may be involving chest wall or breast skin, or both. In this stage, the cancer has also extended to the lymph nodes below or above the collarbone and may also have spread to axillary lymph nodes or to lymph nodes near the breastbone.

Stage IV: This stage describes an invasive breast cancer which has extended outside the breast and adjacent lymph nodes and has affected other organs of the body e.g., lungs, bones, brain, liver, skin etc.

3. Epidemiology of breast cancer

In Modern world, occurrence of non-communicable diseases is increasing day by day [2, 3]. This is mainly due to factors like increased lifespan, prolonged exposure to risk factors and changes in lifestyle. While being one of the most crucial diseases in the world, cancer is also regarded as a complicated-on account of being multi-factorial, epidemiologically. In 2012 alone, around 14.9 million new cases of cancer were tradition, culture, food habits, intra-community marriages and ethnicity. In recent past recorded. It is estimated that in the next two decades, this number will be around 22 million [4]. Now a day, breast cancer is becoming more common in women and is cosmopolitan in nature with high rate of incidence [5]. It accounts for 25% of all types of cancers, recording 1.7 million new cases per year. It is also the second common most cancer [4]. As per WHO, the latest incidence rate of breast cancer in east Africa to west Europe ranges from 19.4 to 89.7 per one lakh people respectively [6]. Other than its fast growth rate in South America and Africa, breast cancer incidence in on rise in several Asian countries too. For instance, Japan witnessed a 6% increase per year from 1999 to 2008. In Australia, mortality rate due to breast cancer has reduced by 2%. While it is increasing in several countries. Malaysia and Thailand recorded the highest increase. The ratio of mortality and incidence of breast cancer in the world and Asia-Pacific countries is 0.30 and 0.27 respectively. However, this ratio of mortality and breast cancer incidence is 0.2 and 0.41 in the Pacific and Southeast-Asian countries respectively [7]. Not so long ago, breast cancer incidence was rare in South Korea. However, now, the incidence and death rate from breast cancer has increased [8]. Hong Kong has seen a decline in the incidence [9]. Generally, different regions show different breast cancer incidence due to difference in risk factors, level of education, different life expectancy, screening programs [10], and cancer registration [2]. The number of diagnosed breast cancer cases is increasing because of the increased life expectancy and increased full health screens [10]. Cancer accounts for around 3–4 million deaths worldwide annually. Of these, 2–3 million deaths occur in developing countries [11]. In India, cervical cancer has a higher occurrence than breast cancer. On the contrary, our state (J&K) in the Indian Subcontinent, Kashmir shows a reverse trend (World Health Organization, 1978) (**Figure 1**). Rise in breast cancer in Kashmir valley is considered a major health concern. Experts attribute this increase in breast cancer to various factors like sedentary lifestyle, bottle feeding, late

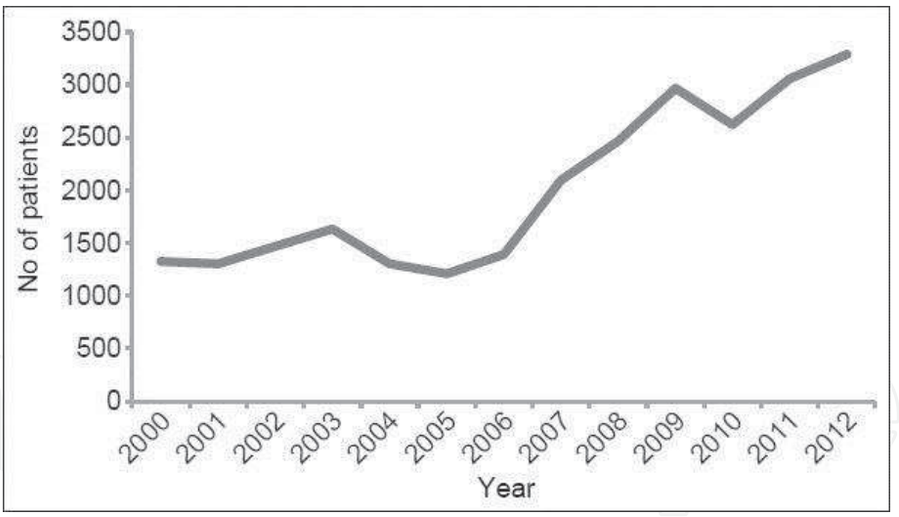

Figure 1.
Shows year-wise number of registered cancer patients, incidence sites and cancer trends 2000–2012). Adapted from Wani [12].

marriages etc. Kashmir valley is quite different from other areas with respect to its unique geographical location, Kashmir has seen a huge increase in the occurrence of breast cancer among its unexplored ethnic population. The overall cancer incidence in Kashmir region is increasing. In men, esophagus and gastroesophageal (GE) junction, lung, stomach, colon, rectum, lymph nodes, skin, laryngopharynx, blood, prostate and brain are the common sites of cancer. While in females the common sites are breast, esophagus and GE junction, ovary, colon, rectum, stomach, lung, gallbladder, lymph nodes, blood and brain.

4. Connexin 43 and breast cancer

Connexin 43 is the most widely expressed gap-junction protein in normal breast tissue and is thought to play important role in normal mammogenesis, lactogenesis and involution [13]. Cx43 is not expressed in normal breast stem cells but is expressed in the normal breast epithelial cells derived from these breast stem cells [14–16]. Studies have shown that Cx43 is down regulated at the mRNA and protein level in human breast tumors and several human mammary tumor cell lines [17]. Decreased expression of connexin gap junctions is seen in breast cancer at various stages of progression and restoration of gap-junction intercellular communication. Studies have shown that Cx43 is down regulated at the mRNA and protein level in human breast tumors and several human mammary tumor cell lines [17]. Decreased expression of connexin gap junctions is seen in breast cancer at various stages of progression and restoration of gap-junction intercellular communication. Under in vitro conditions it has been seen that the upregulation of connexins restore normal phenotype and reduce tumor growth in vivo conditions [18, 19]. Various studies have shown that down-regulation of Cx43 plays role in primary tumor formation as well as its metastasis in breast tissue. Primary breast cancer is generally composed of tumor cells and surrounding connective tissue. The arrangement within cancer creates multiple patterns of cell–cell interactions among tumor cells and between tumor cells and normal neighboring stromal cells. Among, these patterns the GJIC involving Cx43 is considered to be initial step associated with malignant cell transformation. Studies have shown down-regulation of Connexin 43 gap-junction occurs in human breast cancer tissues compared with the non-neoplastic breast tissue surrounding primary tissue. It has been seen that re-expression of Cx43

reverses the malignancy of human mammary carcinoma cells [19–21]. In, rat mammary carcinoma induced by DMBA data obtained demonstrates that connexin 43 gap junction loss is a common feature of transformed mammary neoplastic cells. Furthermore, data obtained with rat mammary carcinoma, induced by DMBA also demonstrates that the loss of connexin 43 gap junction is a common feature of mammary neoplastic transformed cells. In human mammary carcinoma (MDA-MB-435) cells it has observed that the Cx43 re-expression suppresses the cancer phenotype, increases ability of cells to differentiate into well defined 3D structures and also reduces the tumor growth in animal models [20, 22]. Studies have also shown that down-regulation of endogenous connexin 43 expression by small interfering RNA promoted a more aggressive phenotype in human breast cancer cell lines. It was seen in this study that Cx43 reduced the expression of fibroblastic factor receptor (FGFR) and of other proteins that are involved in tumor progression. Studies have revealed that over expression of Cx43 in tumor cells not only restores growth control, but they also revert to less malignant phenotypes [18]. Upregulation of Cx43 by the drugs like genistein and quercetin leads to GJIC-independent inhibition of cell proliferation [23]. Cx43 plays role in tumorigenesis probably by inhibiting angiogenesis independently of cell communication as inhibition of Cx43 expression by RNAi in breast cancer Hs578t cells, resulted in faster growth and increased aggressiveness of the cells, TSP-1 expression was reduced while pro-angiogenic factor VEGF was upregulated. Similar results were observed in MDA-MB-231 cells over expressing Cx43. In addition conditioned media from these cells inhibited in vitro endothelial cell tubulogenesis and migration [24]. Additionally, tumor angiogenesis was decreased in xenografts of Cx43-overexpressing MDA-MB-231 cells. Altogether these findings suggest that Cx43 plays tumor suppressing role by mediating cell proliferation, migration and angiogenesis, enduring support to relatedness between physiological variation in Cx43 levels and aggressive of breast cancer. Tumor metastasis, a multi-step process involves the entry of transformed cells into the circulation after dissociating from the site of origin, extravasation from the vascular system and proliferation into tumor masses at secondary tissue sites. Different stages of this metastatic cascade depend both on cell–cell and cell-matrix interactions [25]. Metastasis has been shown to be promoted by down-regulation of connexins as the breast metastasis suppressor 1 gene exogenous expression in MDA-MB-435 cells led to upregulation of Cx43 and restoration of GJIC, providing evidence that connexins act as tumor suppressors in metastasis [26]. The migratory potential and ability to invade through basement membrane matrix was found to diminish in cells with exogenous expression of Cx26 and Cx43 during functional in vitro studies [27] along with a slight drop-off in matrix metalloproteinase activity [28]. Additionally, studies show decrease in the number of metastases to lungs in mice injected with breast cancer cells expressing Cx43 relative to mice injected with vector controls only [20]. The movement of cancer cells across the endothelial cell barrier as they move in and out of the blood vessels has been shown to be a key step in metastasis and studies have shown that Connexins play an important role in tumor cell vascular intravasation and extravasation [29]. Co-culturing of endothelial cells with breast cancer cells results in the reduction of GJIC in endothelial cells. This reduction weakens cell–cell contacts and also it becomes easier for cells to cross endothelial barrier during the process of extravasation and intravasation [30]. This fact was supported by another study which shows that over expression of Cx43 in HBL-100 breast cancer cells (GJIC deficient) makes them capable of forming heterocellular junctions. These junctions allow dye transfer between human microvascular endothelial cell expressing cx43 and breast cancer cells resulting in tumor cell diapedesis. Treatment of endothelial culture with GJIC inhibitors or co culturing of endothelial cells with

breast cancer cells that express mutated or non-functional Cx43 results in blockage of trans endothelial migration [31]. Hence these studies imply that both homocellular GJIC between endothelial cells and heterocellular GJIC between endothelial cells and breast cancer cells facilitate trans endothelial migration. A series of studies performed on the effects of metastatic breast cancer cells on osteoblast differentiation with MDA-MB-231 and MC3T3-E1 cells showed inhibition of osteoblast differentiation by conditioned medium from breast cancer cells [32, 33]. It has also been demonstrated by other studies that in MDA-MB-231 Cx43 expression results in decreased expression of OB-cadherin [34] a similar trend was also found in Cx43 expressing MDA-MB-435 cells. Decrease in the expression of N-cadherin, a protein which is involved in increased motility, invasion and metastases of breast cancer cells [35, 36] has been observed in in Cx43 over expressing MDA-MB-231 cells [34] this clearly shows that it contributes to decreased metastasis in vivo. In human glioblastoma cells it has been seen that Cx43 enhances response to chemotherapeutic agents or low serum hence confirming the fact that Cx43 shows anti metastatic effect [37]. In human breast cancer tissue, studies have also demonstrated that expression of Cx43 is directly correlated with the expression of BAK (Bcl-2 homologous antagonist/killer), a pro-apoptotic gene of the Bcl-2 family [38]. In human mammary carcinoma cell, MDA-MB-435 Cx43 suppressed the cancer phenotype and cell growth in culture and in animal models. There remains little doubt that down regulation of Cx43 plays a very important role in the primary tumor formation and its metastasis in mammary glands. However, the mechanism by which Cx43 down regulation occurs in breast cancer is far from clear. Several mechanisms like Cx43 promoter hyper-methylation or a cancer-specific reduction of Cx43 expression/trafficking by the modulation of various components of the Cx43 life cycle appear to be involved in the down regulation of Connexins in mammary glands, but irreversible mutational alterations have not yet been proved to be among them. Therefore, the role of Cx43 in carcinogenesis requires further investigations. Additional studies on Cx43 in different cancers, thus, will establish its role in cancer signaling and thus as a therapeutic target.

5. Regulation of Connexin 43 by epigenetic mechanisms and transcription factors

Tumors and transformed cell lines generally exhibit down regulation of Connexin expression. This down regulation is said to be responsible for the loss of proliferating control. However, deletion or mutation of connexin gene as a common factor in human tumors has not yet been demonstrated by various intensive studies on the subject. On the other hand, what various studies have shown is that silencing of Connexin expression in several kinds of malignant cells can be caused due to epigenetic inactivation of the promoter region by hypermethylation. Studies have also indicated that types of cells and connexins determine the effects of DNA methyltransferase inhibitors on connexin expression, as illustrated by Vinekn et al. in a review [39]. A correlation was established with micrometastasis into lymph nodes and the lack of Cx43 mRNA expression in adjacent normal lung cancer tissue in human non-small lung cancers [40]. Patients lacking Cx43 mRNA possessed higher frequency of promoter methylation compared with Cx43 mRNA-positive patients, as reported by Chen. Their data also indicates a possible interference of promoter methylation with AP-1 binding to the promoter which results in lack of Cx43 gene expression. The human Cx43 proximal promoters possesses several binding sides for Sp1 and AP1 transcription factors and have been demonstrated to be indispensable for optimal promoter activity by promoter/report assays and

Sp1/AP1 over expression studies. The Sp1- and Ap1- binding sites were shown to contribute to the activity of the promoter. Each of them also contributed to bind the transcription factors Sp1/Sp3 or AP1, respectively. Both Sp1 and Sp3 resulted in the rat Cx43 promoter activation during trans-activation assays. These findings indicate the importance of the transcription factors Sp1, Sp3 and AP1 in rat Cx43 proximal promoter activity. Cell type-specific expression of Cx43 may thus depend on additional activators or repressors in different Cx43-expressing cell types (including cardiomyocytes) as similarities exist in proximal promoter regulation by universally expressed transcription factors (Sp1, Sp3, AP1). Although the mechanism Connexin gene silencing by DNA methylation is clear, the origin of this epigenetic modification still remains elusive. In liver cancer, elevated DNMT1 mRNA levels are thought to decrease expression of connexins, in casu Cx26 [41]. Moreover, the aberrant binding of transcription factors to methylated Connexin gene promoters may contribute to poor Connexin expression in cancer cells. This is supported by the decreased Cx43 gene transcription accompanied by DNA methylation in human non-small cell lung cancer cells. The decreased Cx43 gene transcription is also correlated with reduced binding of activator protein 1 (AP1) to its promoter [40]. Furthermore, in human breast cancer cells [42] and rat liver cancer cells [43] the Sp1 cis-acting elements of the Cx26 and the Cx32 gene promoter are especially rich in methylated CpG dinucleotides.

6. Regulation of Connexin 43 by micro RNAs

Almost one-tenth of all new cancers and 23% of cancer cases detected in females are breast carcinomas with more than 1 million diagnoses every year worldwide [44, 45]. Major causes of this disease-related death are relapse and metastasis [46, 47]. Recent studies that on the metastatic mechanisms of breast cancer suggest the gap junction to be a major regulator of tumor metastasis [48]. Located at the cell membrane, the gap junction primarily comprises of different connexin proteins. These connexin proteins are closely associated with numerous functions of the cell [49, 50]. Connexins constitute a family of 21 members with Cx43 being abundantly expressed in the mammary gland [49]. It is reported that Cx43 plays an important role in normal cell migration [51] and tumor cell invasion [52]. As such, promising strategies in regulating cell functions are provided by the regulation of Cx43 expression [53, 54]. Different transcription factors tightly regulate the expression of CX43 gene at transcription level. Studies have found that Sp1 (specificity protein 1), Sp3, AP-1 (activating protein 1) and c-Jun can promote transcriptional activity of Cx43 gene by directly binding to its promoter [55, 54] addition, at the post-transcription level Cx43 is also closely regulated by miRNAs [53, 56, 57]. miRNAs, largest groups of posttranscriptional regulators, [58]. Eight bases at the 5′end of miRNAs, are involved in posttranscriptional regulation. These two to eight bases could bind to the 3′-UTR of the target genes in order to bring about inhibition of gene expression at mRNA level [58]. By virtue of their direct or indirect regulation of target gene expression, miRNAs regulate a number of biological processes. The processes include cell cycle [59], growth [60], apoptosis [60], differentiation [61] and stress reaction [62]. Studies have identified miR-1, miR-206, and miR-381 as potent suppressors of Cx43 [53, 56, 63]. Cx43 has been found to enhance metastasis in breast cancer cells. It has been proven to be a direct negative target for miR-206, miR-1 and miR-133 and an indirect target for miR 381 [8]. During the myoblast differentiation in vitro and in vivo, two related miRNAs, miR-206 and miR-1, cause inhibition of Cx43 protein expression without altering Cx43 mRNA levels [63]. Further it has been reported by Anderson et al. that Cx43 mRNA contains

two binding sites in its 3'UTR for miR-206/miR-1, both of which are essential for an efficient down regulation. Also, they observed sections of eight nucleotides in the 3'UTR of Cx43 gene that are complementary to the first eight nucleotides from the 5′ end of miR-1. Which then they proved that miR-1 binds to these nucleotide sequences. miR-1 was also shown to cause reduction of Cx43 levels in isolated neonatal rat ventricular myocytes in culture [64]. They further found two putative target sequences in the 3′ UTR of Cx43 for miR-206 and proved that miR-206 that is expressed ectopically binds these sites. Moreover, the ectopic expression of miR-206 downregulated the endogenous expression Cx43 gene without affecting Cx43 mRNA expression. The continuous expression of miR-206 in osteoblasts resulted in decreased expression of osteoblast differentiation and Cx43 protein expression. The suppression of Cx43 gene expression was caused by miR-381 via the promoter region −500/−250 miR-381 could directly bind the sequences CACUUGUAU in the 3′UTR. Site-directed gene mutation was done (CCAAT/enhancer-binding protein α) in order to inhibit C/EBPα expression. By binding it to a canonic element (AATTGTC) located at −459/−453 in the promoter region of the Cx43 gene, they identified C/EBPα as a novel transcription factor. Therefore, miR-381 causes C/EBPα dependent Cx43 suppression in breast cancer cells.

7. IRES mediated regulation of Connexin 43

Connexin 43 (Cx43) is one of the main gap junction (cell–cell channel) proteins expressed in the heart ventricle. Constitutive expression of Connexin 43 has been found to be responsible for the anisotropic propagation of action potentials in the heart [65]. And also, Cx43 gap junctions are essential for the synchronous contraction of the myometrium of the uterus during labour pain. While the expression of Cx43 is ordinarily sparse in the myometrium, the ovarian hormones and mechanical stretch upregulate it [66]. This upregulation is seen at the transcriptional as well as the translational level, as there is accumulation of Cx43 mRNA before the swift advent of Cx43 protein, just prior to childbirth [67–70]. The Cx43 gene like most of the connexin genes consists of two exons separated by a large intron. Exon 1 contains most of the 5P-untranslated region (5P-Utr) while the remaining 13 bases of the 5P-UTR followed by the entire coding region and the 3P-UTR are contained in Exon 2. There is wide acceptance of observation that in eukaryotes, protein synthesis initiation begins with the binding of the small ribosomal subunit to the 5P-cap structure. Then the mRNA is scanned by the 40S ribosome until it encounters an AUG codon where the 60S ribosomal subunit joins, and hence the translation begins. Between the cap structure and the first AUG codon, most cellular mRNAs contain fewer than 50 nt between the cap structure and the first AUG codon but the 5P-UTR of Cx43 mRNA has been found to 208 nt. In addition, the 5P-UTR of Cx43 mRNA has a stable secondary structure. The scanning of the 40S ribosome can be inhibited by such structures. The secondary structures of the Cx43 IRES and most of the other described IRES elements have a semi-conserved Y-like structure, which is suggested to have role in the IRES mediated translation in eukaryotic cells in Stress conditions. Inhibition of cap-dependent translation is one of the cellular responses to stress [71]. This inhibition allows continuation of synthesis of proteins essential for survival and stops the synthesis of non-essential proteins. Illustrations for this are as follows: VEGF is translated in response to hypoxia [72], the translation of the chaperone proteins Bip [73] and hsp70 takes place under conditions of cellular stress in response to misfolded and degraded proteins and in the infarcted myocardium FGF-2 functions in the salvage of cells [74]. In all these genes, IRES elements have been found that are translated even under stress conditions. The need

is to maintain intercellular communication via Cx43 channels even under certain stressful conditions likely. For instance, in the hypoxic hear gap junctional remodeling occurs [75] requiring the synthesis of new Cx43. Recent reports have claimed that in addition to estrogen, mechanical stretch is required to upregulate expression of Cx43 in the uterus at the commencement of labor [66]. The fetus grows faster than the uterus during the later phase of pregnancy which causes physical stretch in the myometrium. As such, Cx43 must be speedily upregulated during this time. A mechanism by which a high level of translation can be accomplished during this stress may be offered by the IRES.

8. Carboxy terminal domain of Connexin 43 and human breast cancer

Cellular communication is paramount for tissue/organ homeostasis in multicellular organisms. Exchange of small ions, secondary messengers and small metabolites required for electrical and bio-chemical coupling between cells is mediated via intercellular channels known as gap junctions [76, 77]. Each gap junction is formed by association of Connexin proteins. Human genome contains 21 different Connexin genes, expressed differentially in various types of cells and tissues [78]. Among these gap junction proteins, connexin 43 (Cx43) is major gap junction protein which is widely expressed across tissues and besides its role in mediating cell to cell communication, it also plays very critical role in cellular proliferation [79]. More precisely, Cx43 acts a tumor suppressor [80] usually downregulated in various diseases such as cancer [81, 82], connexin 43 possesses long cytosolic C-terminus and most of the non-canonical functions of connexin 43 are attributed to it [83] (**Figure 2**).

More interestingly independent of full length Cx43, CT-Cx43 expression has been found to occur in various cell types [85]. This CT domain is subjected to various post translational modifications like phosphorylation, S-nitrosylation and

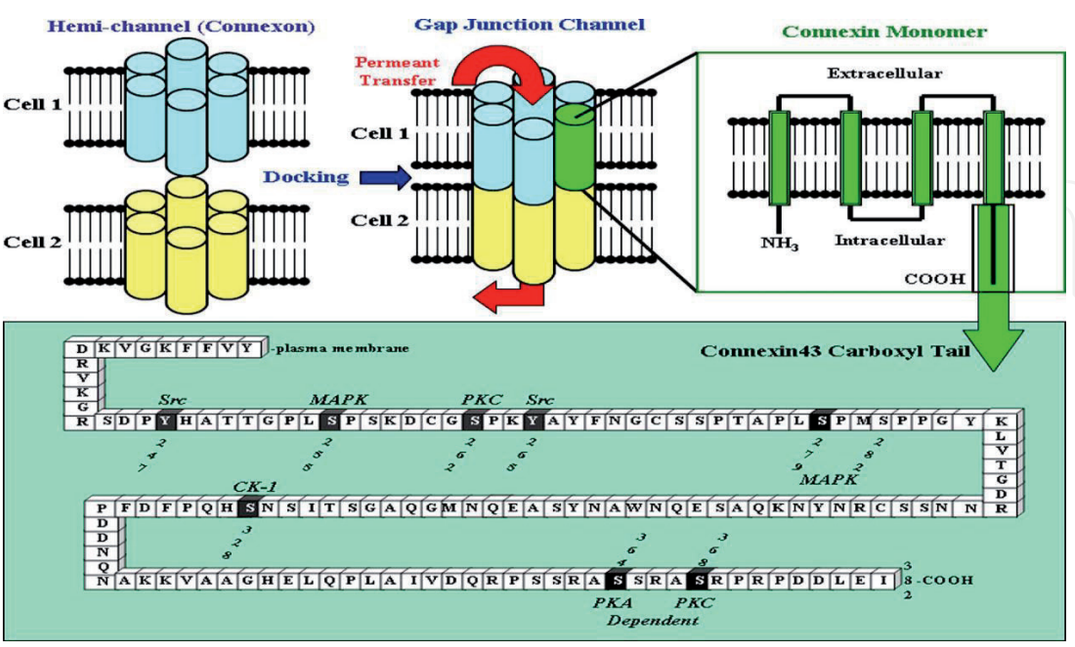

Figure 2.
Gap junctional intercellular communication (GJC) mediated by connexin proteins. Hexameric arrangements of connexin monomers comprise a hemi-channel or connexon. Adjoining cells each contribute one connexon to form a complete gap junction channel. For several connexin types, the assembly, gating and turnover of this channel are regulated to a large extent via phosphorylation of the cytoplasmic tail by various cellular kinases including: Src, PKC and MAPK. Adapted from (king and Bertram, 2005) [84].

truncation [86]. Also CT-Cx43 has been shown to interact directly and indirectly with microtubules and actin respectively. The later takes place by the interaction of CT-Cx43 with adaptor proteins such as zonula occludens-1 (ZO-1) and drebrin (developmentally regulated brain protein). Perturbation of this interaction has been implicated for the development of various developmental and cardiac defects (**Figure 3**) [87].

The growth suppressing effect of Cx43 was not compromised while expressing only CT-Cx-43 in HeLa cells [89] and HEK-293 cells [89]. CT-Cx-43 has been shown to have nuclear localization implying that it may be involved in regulating gene expression directly or indirectly within the nucleus [89]. In direct regulation it may act as a transcriptional activator or repressor of target genes however, in indirect regulation it may regulate target gene by acting as a transcriptional activator or repressor of miRNAs targeting them. In various cancers it has been shown the expression of one tumor suppressor gene can rescue the expression of other tumor suppressor gene as well [75, 90]. However the exact mechanism is not fully understood. P53 known to act as guardian of genome is a tumor suppressor playing very important role in regulating cellular process [91] such as cell proliferation [92, 93]. The expression of p53 increases under stress conditions [94] than its basal levels under normal condition [95]. These stress conditions include DNA damage [96] and oncogenic insults [97]. Dysregulation of p53 is considered to be initiator of tumorigenesis which includes its down regulation or mutation [98–100]. Expression of p53 has been found to be upregulated by CT-Cx43 in cardiomyocytes [88]. In addition, a group of small RNAs, i.e., microRNAs (miRNAs), has been shown to be able to regulate the expression of genes implicated in various normal and pathological conditions, including cellular proliferation and cancer [101–106]. More precisely a conserved homolog of *C. elegans* miRNA lin-4 namely miR-125b has been found to be dysregulated in various cancers [107]. The expressional studies of miR-125b in various cancers have revealed that miR-125b is upregulated in some cancers and

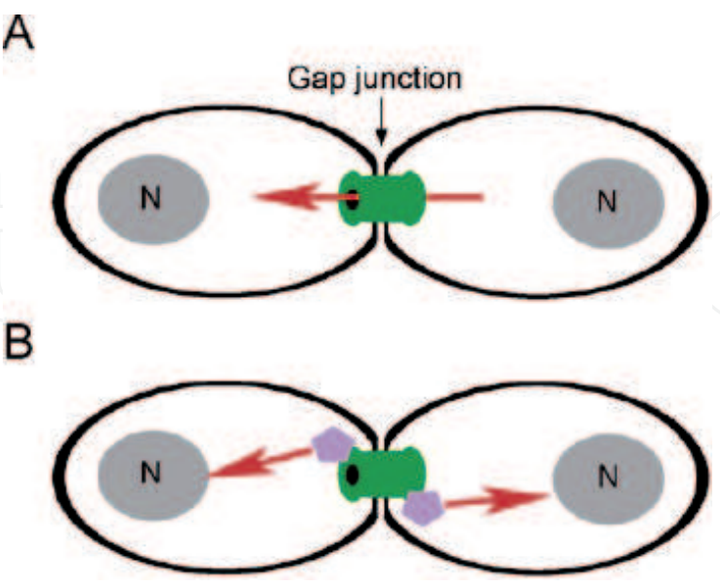

Figure 3.
Channel dependent and independent mechanisms by which Connexin expression can alter other genes. (A) Channel dependent mechanism. In this model, signaling molecules (red arrow) are directly exchanged between cell cytoplasms there by coordinately regulating gene expression patterns in the nucleus (N) (B) connexin dependent but channel independent mechanism. In this model connexins that may or may not be at junction membrane either bind a molecule with transcriptional activity (purple trapezoid) or can cleave such a portion of carboxy terminus to signal to the nucleus In this model connexins that may or may not be at junction membrane either bind a molecule with transcriptional activity (purple trapezoid) or can cleave such a portion of carboxy terminus to signal to the nucleus. Adapted from (Kardami et al., 2007) [88].

downregulated in others such as breast cancer. Therefore, it has occasionally been labeled as a tumor suppressor. Most of the dysregulated miRNAs has been shown to target tumor suppressor genes such as PTEN, RB, Cx43 and p53 [108, 109].

9. Connexin 43 as theurapatic target

Connexins have a dynamic role in the metastatic process, involving multiple factors. Metastasis is preceded by a series of events -tumor cells leave the primary tumor, move too far off sites and some start secondary tumors. While dealing with therapeutic issues, this variety of roles of connexins and GJIC in tumor development requires special attention. The anti-tumor growth function of connexins and their observed loss in cancer has made it clear that a possible strategy to inhibit tumor growth could be provided by restoring connexins expression. The targets of anti-cancer therapeutics are enzymes that affect global gene expression including HDAC, a set of enzymes involved in chromatin remodeling. Upon generation and testing of many HDAC inhibitors (HDACi), it has been observed that the effects of these drugs (at least some part of them) are GJIC-dependent. When prostate cancer cells were treated with the HDACi Trichostatin A (TSA) restoration of both Cx43 expression and GJIC takes place [110]. Hyperphosphorylation and degradation of Cx43 was also countered via the modulation of MAP kinases and Src [111]. Proteins that are involved in tumor progression and metastasis can regulate Connexin expression at transcriptional level. Cx43 is transcriptionally upregulated by the Ras–Raf-MAPK pathway via the interaction of its promoter with a protein complex that contains both HSP90 and c-Myc [112]. Protein AML1-ETO fusion protein transcriptionally upregulated Cx43 expression resulting from the chromosomal translocation t(8;21) frequently associated with acute myeloid leukemia (AML) via the JNK signaling pathway. The JNK specific inhibitor SP600125 was shown to inhibit this effect [55]. Melanoma metastasis was promoted by the protease-activated receptor-1 (PAR-1) via transcriptional regulation of Cx43 [54, 113]. The importance of Connexin phosphorylation, especially Cx43, in the regulation of their levels and functions has been extensively investigated [114]. The stability and degradation of Connexin proteins are regulated by the lysosomal and proteasomal systems, in addition to phosphorylation [113]. The efficacy of drugs could be improved by the stabilization of the Cx43 protein. For example, while sensitizing cells to the pro-apoptotic effect of MG132, the rate of degradation was decreased by treatment with the proteasome inhibitor MG132 [115]. A major regulatory event in the life of Connexins is phosphorylation by the kinase Src. Phosphorylation by Src takes place either directly or via signaling intermediates such as PKC and MAPK which resuls in a disruption of GJIC [116]. This effect has been shown to lead to drug resistance [117]. In colon cancer cells that already express Cx43 mRNA, Cx43 expression and phosphorylation were enhanced by Kaempferol, a plant flavonoid, via a Stat3-dependent mechanism. However, Kaempferol showed no effect in cells that were devoid of Cx43 mRNA and deficient in GJIC [118]. Therefore, targeting the post-translational modification of connexins is limited by the requirement of a functional transcriptional regulation. Such an isolated treatment would not be useful unless in combination with other treatments such as methylation- or acetylation-modulatory agents in order to unblock the transcription of c bvonnexins. In ovarian cancer cells, Cx43 phosphorylation and inhibition of GJIC was brought about by their treatment with endothelin-1 (ET-1), a ligand for the ETA receptor (ETAR), which is overexpressed in ovarian carcinoma [119]. The selective ETAR antagonist BQ 123, the tyrosine kinase inhibitor tyrphostin 25 or the c-Src inhibitor 4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo [3, 4-d] pyramidine (PP2) blocked this

effect, which suggests a role for Src in this mechanism [119]. Further, inhibition of ovarian tumor growth in vivo alongside a reduction of Cx43 phosphorylation was caused by ABT-627, an ETAR antagonist [119]. Summarily, the efficacy or specificity of these drugs can be increased by a combinatory approach considering an effect on both the Connexins and their regulatory molecules. In conclusion, gap junctional intercellular communication mediated by Connexins offer immense therapeutic opportunities that are still widely open. This approach is supported by original tools in the form of new findings regarding the regulation of Connexins expression. In view of the vast array of data about Connexins generated in various different tumor models and contexts, it is perhaps the right time for a consensus meeting devoted to focusing attention of the possibilities for Connexins as therapeutic targets.

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