2. Review of Literature

2.1. Breast cancer incidence

Cancer is a disease caused by uncontrolled proliferation of cells. The cell which loses the control over its regulation of cell cycle can also invade the surrounding tissues leading to malignant growth. There are several types of cancers being the major cause of death worldwide. Breast cancer is the second most common cancer in women worldwide.

2.1.1. Breast cancer incidence Worldwide

Breast cancer is the second most common form of cancer worldwide. More than 1.3 million women worldwide are diagnosed with breast cancer each year [24]. Breast cancer rate has increased by 0.4% per year from 1975-1990. Later, breast cancer death rates decreased by 2.2% per year between from 1990-2007. However, not all segments of the population have been benefited from these advances. For example, Breast cancer rate has remained unchanged among American Indian/Alaska Natives [25]. In spite of improved breast cancer rate about half-a-million women still die because of breast cancer each year [24].

About 1.3 million women in the world are diagnosed with breast cancer annually and about 465,000 die from the disease [26]. According to American cancer society, it was estimated that 231,840 new cases of invasive breast cancer will be diagnosed among women, as well as an estimated 60,290 additional cases of in situ breast cancer will be diagnosed leading to 40, 290 death in US [1]. Every year nearly 60,000 people are diagnosed with breast cancer in the UK, that's the equivalent of 1 in 8 women in the UK will develop breast cancer in their lifetime [27]. Reports suggest that in global population, Asian countries represent 59% of the global population, accounted for 39% of new cases, 44% of deaths, and 37% of the world's 5-year prevalent cases. Although North America (US and Canada) represents only 5% of the world population, it accounted for 15% of new cases, 9% of deaths, and 17% of the prevalent cases. In contrast, African countries (15% of the world population) represented 8% of the total new cases and 12% of breast cancer deaths, and 7% of the prevalence [28]. European countries represent 29% of new breast cancer cases and 25% of deaths and 29% of the prevalence [28]. China comprises of the total of 11% breast cancer cases of the total cases diagnosed worldwide [29].

2.1.2. Breast cancer incidence in India

The incidence of breast cancer in India is rising due to the lack of awareness and the absence of a proper breast cancer screening program. The majority of breast cancers are diagnosed at a relatively advanced stage [30]. The highest rate of breast cancer is seen in northeast and metropolitan cities such as Delhi, Mumbai, Ahmedabad, Calcutta, and Trivandrum where it constitutes > 30% of all cancers in females [31, 32]. The variation is because of the difference in education, reproductive, adiposity and varied lifestyle [33].

The high figures of incidences and mortality, even with the advancement of primary screening and diagnosis, suggest the need to systematically investigate the cause and pathogenesis of breast cancer. Due to high mortality rate, extensive efforts have been made to understand the mechanisms involved in the pathogenesis of breast cancer and identify potential therapeutic targets. In general, cancer arises due to failure of the mechanisms that control cell growth, proliferation and death. Normally, the genetic control system regulates the balance between cell proliferation and death in response to growth inhibitory or stimulatory signals. The mutations or genetic alterations, cell loses its ability to maintain the balance between cell survival and death which leads to uncontrolled proliferation of cells leading to cancerous condition. Although extensive studies have been done to understand the molecular mechanism and processes involved in cancer, further efforts are needed to modulate the signaling pathways to regulate the progression and metastasis of cancer.

2.2. Inflammation and cancer

One of the very essential physiological processes that lead to cancer is inflammation. Association of inflammation and cancer has been studied since long [14, 34-37]. Nearly 15% of the worldwide incidence of cancer is associated with microbial infection. There are several examples showing the linkages between cancer and inflammation as discussed in chapter1. Moreover, *Helicobacter pylori* infection is associated with the development of gastric cancer [38]. Overconsumption of alcohol leads to liver injury (inflammation) that leads to hepatocellular carcinoma. Similarly, cigarette smoking or silica exposure leads to the inflammation in lung and esophagus that ultimately progress into cancer.

2.2.1. Inflammation and breast cancer

There are strong evidences emerging that support the linkages between breast cancer and inflammation [39]. The organ of breast had evolved with primarily two major objectives to provide immunogenic proteins and nutrients to the new born. These proteins provide innate immunity to the new born [40]. Macrophages, an essential component of innate immune system, are in fact permanent resident in the breast tissue [41]. These macrophages whose role is to protect the host against infection facilitate breast tumorigenesis and are known as tumor associated macrophages (TAMs) [20]. Chronic inflammation indeed is reported to play a critical role in breast cancer recurrence [5, 42]. The reports suggest that elevated levels of inflammatory markers such as C-reactive protein (CRP) and serum amyloid A (SAA) are associated with decreased cell survival irrespective of age, tumor stage, body mass index and race. The circulating C-reactive protein CRP and SAA may be important prognostic markers in breast cancer patients [43]. Anti-inflammatory or antioxidants are indeed considered as new therapeutic approach for the treatment of breast cancer [44]. Inflammatory Breast Cancer (IBC) is one of the most aggressive types of breast cancer, suggesting that inflammation might be linked to breast cancer [6, 45].

2.2.2. Regulators of inflammation in breast cancer

2.2.2.1. Pathogen sensing receptors: Linking inflammation to cancer

Inflammation is regulated by different cellular receptors in response to the infectious agent or tissue injury. The regulatory receptors include toll like receptors, RIG like receptors and cGAS (cyclic GMP-AMP-synthase). Different receptors react to different specific ligand. The ligand or stimuli are either exogenous (PAMPs, pathogen associated molecular patterns) or endogenous (DAMPs, Danger associated molecular patterns) [39, 46, 47]. PAMPs are conserved microbial products that interact with receptors to induce immune signaling pathway. The tumor cells undergo continuous oxidative stress that leads to dsDNA breaks that is released into cytoplasm known as DAMPs. The DAMPs may activate TLR signaling (Fig 2.1). TLR9 specifically recognizes dsDNA and activates further downstream signaling process. Mitochondrial DNA that may acts as potential DAMP is known to lead chronic inflammation that subsequently causes heart failure [48]. Emerging evidences also suggest that cGAS (cyclic GMP-AMP synthase) recognizes the

cGAMPs (cyclic GMP-AMP) that are derived from damaged mitochondrial DNA released in cytosol [49, 50]. There is also an alternative TLR independent pathway which involves RIG-I receptor is essential during antiviral signaling [17]. RIG-I recognizes dsRNA to induce inflammatory response [17]. miRNAs are present in the cell that may also act as ligand for RIG-1. RIG pathway also exhibits the similar effect enhancing IFN and NF-κB production. Hence intracellular receptors through NF-κB activation and IFN production seems to be an important link to inflammation and cancer.

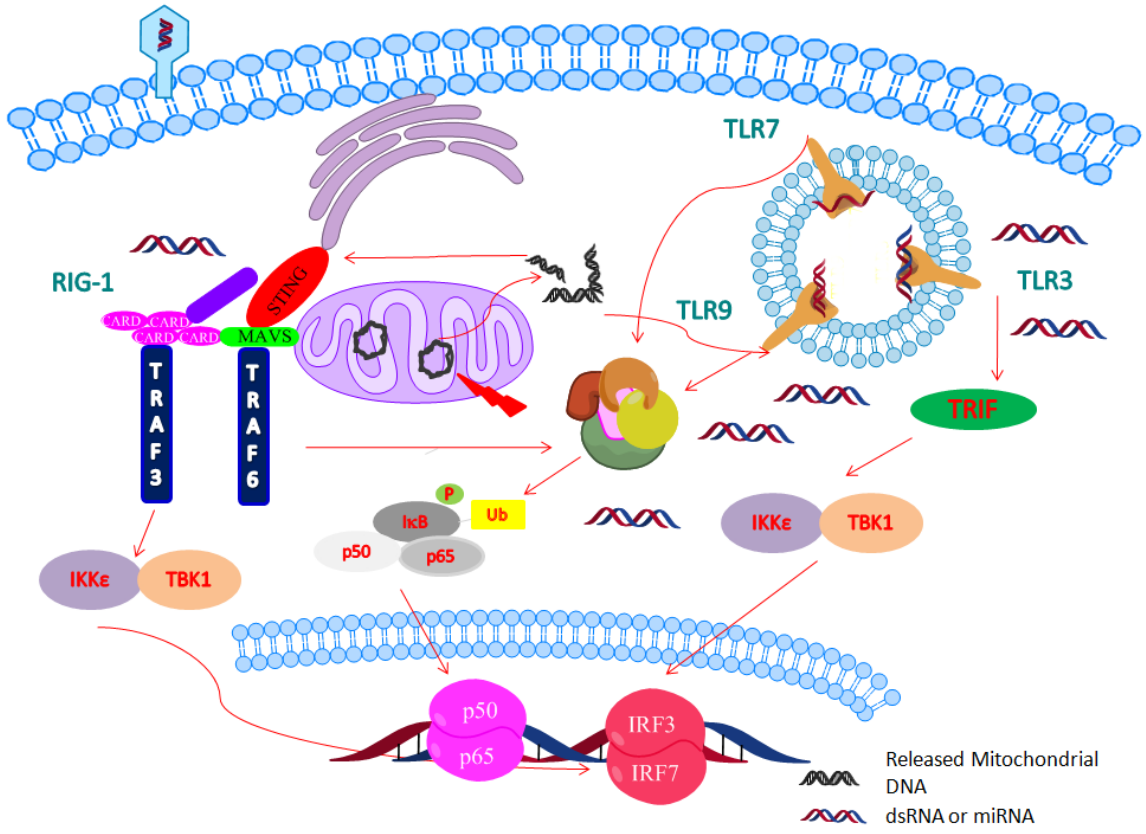

Figure-2.1: Released DAMPs leads to the activation of inflammatory pathways:

The damaged mitochondrial or nuclear DNA may be released into the cytosol. miRNA as well as such released DNA may act as DAMP and activate innate immune signaling pathways [39].

Reports also suggest that these receptors are mutated in several types of cancers including breast cancer. One of the studies reported a very high expression level of TLR3, TLR4 and

TLR9 at mRNA level in patient samples of breast carcinomas [51]. The authors showed here higher TLR expression levels in tissue samples from patients with recurrence relative to samples without recurrence. The authors observed the expression of TLRs generally on cancer cells but also on some stromal cells. The study also revealed that TLR3 expression by tumoral cells and TLR4 expression in mononuclear inflammatory cells is associated with higher rate of distant metastasis and TLR9 expression on fibroblast like cells showed low rate of distant metastasis.

MDA-MB-231, an epithelial breast cancer cell line derived from metastatic site pleural effusion, mimics the breast cancer cell properties in vitro. Hence it is used as a tool for the ease to study breast cancer at genetic level. It has been observed that MDA-MB-231 expressed all the TLRs at different level whereas TLR3 expression was least. In comparison of TLR3, TLR4 was expressed 5 fold higher and other TLRs were in range of 1-4 fold higher than TLR3 [52]. Yang et al. showed moderate expression of TLR4, TLR5, TLR6 and TLR7 and others at low level or completely absent. Xie et al, used MDA-MB-231 cell line, where TLR2 is highly expressed, and showed that the high invasiveness property of the cell line is because of the TLR2 over expression. The study also showed that TLR2 activation enhanced the activity of NF-κB and induced phosphorylation of TAK1 and IκBα as well as IL-6, TGF-b, VEGF and MMP9 secretion, which are associated with TLR2-NF-κB signaling [53]. Knockdown of TLR4 gene has also shown to reduce breast cancer cell viability in MDA-MB-231 cell line [52]. Similarly TLR9 activation by TLR9 agonist rendered MDA-MB-231 cells highly invasive [54]. MCF7 an epithelial cell line is another very good model to study cancer in vitro. TLR3 mRNA level was found to be significantly high in MCF-7 cell line and it was also found to express TLR1, TLR2, TLR5, TLR6 and TLR8 at different level [53].

However, TLR3, TLR5 and TLR7 shows opposing effect on the survival of cell lines, where they are present. TLR5 when activated by flagellin inhibits cell proliferation in MCF7 cells as well as in mouse xenografts of human breast cancer cells by activating expression of CDK inhibitor p27 and decreasing cyclins and other soluble factors. TLR3 ligation with synthetic dsRNA induces apoptosis of human breast cancer cells in a TLR3-dependent manner, which involves the molecular adaptor Toll/IL-1R domain-containing adapter inducing IFN-β and type I IFN autocrine signaling in Cama-1 cell line [55].

Similarly, TLR7 agonist, imiquimod, can elicit significant regression of spontaneous breast cancers in neu transgenic mice, a model of human HER-2/neu+ breast cancer [56]. Thus, the TLRs have profound effect on breast cancer and silencing or activating them might prove to be an effective therapeutic target for inhibiting the breast cancer. Though patient sample data and studies on breast cancer cell line contradict in case of TLR3 and TLR9, further study is needed to understand the TLR signaling mechanism in cancer cells.

RIG-I expression is observed to be lower in estrogen receptor (ER) positive breast cancer tissues than in estrogen receptor negative breast cancer tissues [57]. Similarly, RIG-I expression is higher in progesterone receptor (PR) negative cells than progesterone positive. The same is observed in the breast cancer cell lines. MCF-7 has very low expression of RIG-I [57].

cGAS pathway is also emerging as an essential link between innate immunity and cancer [58]. The activation of cGAS/STING pathway by cGMPs shows potential antitumoric activity. cGMPs are now emerging as an essential tool for cancer immunotherapy [59, 60]. Regulators of cGAS pathway such as MITA (Mediator of Interferon Activation) also known as STING is now emerging as tumor suppressor in several cancers types. MITA is downregulated in many of the cancer subtypes and acts as tumor suppressor including in breast cancer [61-64]. Moreover, MITA downregulation diminishes the antitumorigenic potential of cGMPs [59]. Reports also suggest that direct activation of MITA in tumor microenvironment leads to potent and systemic tumor regression and immunity [65].

2.2.2.2. NF-κB and IFNs: Linking inflammation to cancer

There are ways that converts chronic inflammation to the cancerous condition. Pathogen or its component on the surface interacts with the TLRs, toll like receptors on the cell surface of immune cells and induces the signalling that activates the kinase cascade leading to phosphorylation of NF-κB transcription factor. The activation of NF-κB induces the expression of several inflammatory cytokines and chemokines. These cytokines recruits and activates various leukocytes and activates the same transcription factors like NF-κB leading to more cytokine production [66]. It forms a positive feedback loop (Fig-2.2). These cytokines and chemokines favour the process of angiogenesis and

cell migration and favor the tumor growth. Reports show that proinflammatory cytokines like TNFα, IL-6 and IL-10 indeed induces tumor growth [67].

Dysregulation of NF-κB has also been associated with breast cancer. Increased NF-κB activity has been found in both ER (estrogen receptor) positive and ER negative breast cancer patients. IKKε, a central kinase in NF-κB pathway, is amplified and overexpressed in 30 % of breast cancer cell lines and patient-derived tumors [68, 69]. However, the significance and molecular basis of IKKε overexpression in breast cancer is not well studied. DNA damage induces kinase depended IKKε translocation to the nucleus where it gets sumoylated and activates NF-κB [39, 70]. This activation of IKKε might be possibly linked with TLRs. Understanding the mechanism is of great interest and finding the missing links in the pathway as well as modulation of this pathway might open a new era for breast cancer treatment.

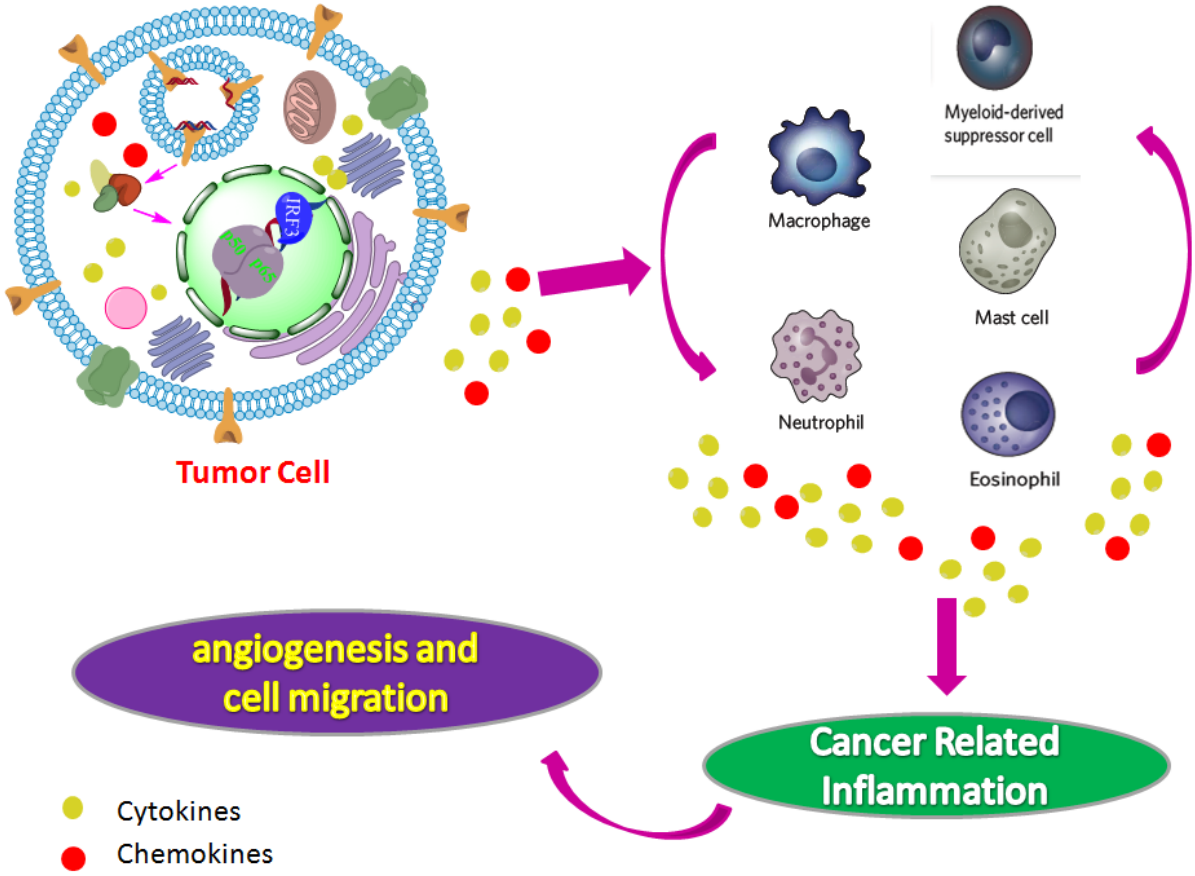

Figure-2.2: Role of NF-κB and IFN in linking inflammation to cancer:

Tumor cells secrete cytokines and chemokines that attract immune cells to the site and mounts cancer related inflammation. This favors the growth and migration of tumor[39].

NF-κB transcription factor stimulates several genes that plays very important role in cell survival (TNF, IL-1, IL-6, cyclin D1, c-MYC), apoptosis induction (Fas, FasL, DR4 (Death Receptor 4), DR5), resistance to the cell death (Bcl-2, Bcl-xL, XIAP, c-IAP1, c-IAP2, c-FLIP), migration (ICAM and VCAM), angiogenesis (VEGF, TNF, IL-1, IL-8) etc (Fig-2.3). ER resident protein MITA (Mediator of IRF3 activation), regulate NF-κB during viral infection. We recently reported that MITA acts as tumor suppressor in breast cancer. It is down regulated in breast tumor tissues. MITA expression in breast cancer cell line activates NF-κB, which in turn induces TNF. MITA also sensitizes TNF induced cell death in breast cancer cell lines, by activating caspases. The activation of NF-κB also increases the levels of pro-apoptotic genes. The mechanisms leading to either cellular proliferation and inhibiting apoptosis resulting in oncogenesis is not well understood.

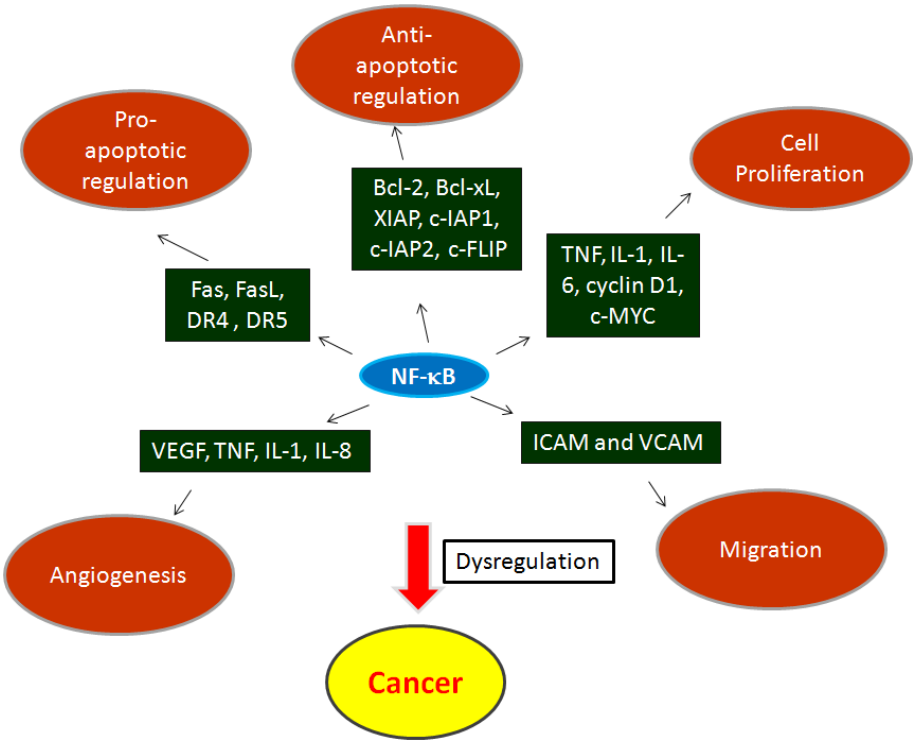

***Figure-2.3 Role of NF-κB in regulation of tumorigenesis*:**

NF-κB activates several genes involved in apoptosis, cell proliferation, angiogenesis etc. Dysregulation of NF-κB leads to cancer condition[39].

NF-κB pathway must be tightly regulated, as a little dysregulation contributes to carcinogenesis. It had been observed that the inhibition of NF-κB leads to the apoptosis of transformed hepatocytes and inhibiting the tumor progression in Mdr2 knockout mice [71]. Endotoxin/lipopolysaccharide, TLR4 ligand, promotes tumor cell adhesion and metastasis by activating NF-κB [72]. High invasive property of MDA-MB-231 cell line, where TLR2 is highly expressed, is because of the TLR2 over expression [53]. TLR2 activation enhances the activity of NF-κB and induced phosphorylation of TAK1 and IκBα as well as IL-6, TGF-β, VEGF and MMP9 secretion, which are associated with TLR2-NF-κB signaling [53]. Thus NF-kB is the central regulator in many of the cancer and its controlled regulation may prove to be an effective cancer therapeutic. Researchers thus find it interesting to study its role and mechanism in cancer.

Type-I IFNs: The first cytokine as anticancer therapy. They are essential in modulating innate immunity during viral infection. They were first discovered as an antiviral agent about 50 years ago, later it was observed to inhibit the growth of tumor cells and inhibit cellular transformation by viruses [73]. IFN such as IFNα is in phase III clinical trial against advanced renal cell carcinoma [74, 75]; there are severe side effects of IFN therapy. IFN therapy leads to autoimmune response in the patients [76]. It also leads to tissue toxicity hence needs to be replaced by targeted therapy. IFNs have short half life time during circulation [76]. The limitations and side effects of IFN therapy suggest the necessity of targeted delivery of IFNs. The possibility to stimulate the cancer cells themselves to produce IFNs can be investigated as another alternative for targeted therapies. TLRs (Toll like receptors) and RLRs (RIG-I like receptors) play critical role in regulation of IFN and NF-κB, hence stimulating these pathways might prove to be an effective IFN treatment. It needs extensive studies to understand the molecular mechanisms involved in these pathways as well as how cancer cells have evolved these mechanisms for their benefits.

2.3. Autophagy: Emerging hallmark of cancer

Autophagy (Macroautophagy) is a sequential process of degradation of cytoplasmic material as well as organelle through lysosomes. The first step involves the formation of autophagophore membrane which encloses the portion of cytoplasm to form

autophagosome [77]. The outer membrane of autophagosomes then fuses with the lysosomes and forms autophagolysosomes [78, 79]. The lysosomal activity leads to degradation of the enclosed cytoplasmic material and inner membrane of autophagosome [80]. Defect in autophagy leads to several diseased conditions such as neurodegeneration, inflammatory diseases and cancer [81]. TFEB (Transcription factor EB) is a transcription regulator of CLEAR network of lysosomal biogenesis [82]. The evidences also suggest that apart from lysosomal biogenesis TFEB also regulates autophagic flux by regulating genes involved in autophagosome synthesis, cargo recognition, autophagosome to lysosome fusion and degradation [83].

2.3.1. Role of autophagy in cancer

Several hallmarks of cancers have been described earlier which also includes inflammation. Autophagy is a major and upcoming hallmark dysregulation of which might lead to cancer. Role of autophagy in cancer is one of the major interests of researchers but a lot remains to be understood about the field. A tumor formation is a slow process of evolution, where a cell down regulates genes specific to growth inhibition and promotes the expression of growth promoting genes. Autophagy is one such process which is very crucial in regulating tumor growth [84]. The cell hence modifies the process during tumor development for its own benefit. Autophagy acts as a dual sword in regulating tumor[85]. In the initial stages of tumor development, autophagy inhibits the process of tumor formation, but at the later stages it provides necessary nutrients to the cells to meet their fast proliferating growth rate [86, 87]. The cancer cells show two kinds of phenotype with respect to autophagy. Few cell types are completely dependent on autophagy *i.e.* if autophagy is inhibited; their growth ceases for example triple negative breast cancer cells [88]. The others need autophagy only under stress. These suggest that autophagy plays very important role in the development of tumor. Tumor must be modifying the process according to their benefit; hence it is essential to study the effect of autophagy in breast cancer.

There are several autophagy regulators mutated in different types of cancers. Beclin-1 is an essential regulator of autophagy induction. The loss of beclin-1 is reported in breast cancer, ovarian cancer and prostate cancer. UVRAG and Bax-interacting factor-1 (Bif-1) are also positive regulators of Beclin-1[89, 90]. UVRAG is reported to be deleted in colon,

gastric and breast cancers [91]. Bif-1, an autophagy mediator, expression is significantly decreased in colon cancer, prostate cancer, urinary bladder cancer, and gastric carcinoma [92]. p53 regulates of autophagy both the ways positively or negatively [93]. It has been observed to be mutated in 50% of the breast cancer. The ATG genes (for example, Atg2B, Atg5, Atg9B, and Atg12) are also mutated in several cancer patients and cellular or animal models of tumorigenesis [94, 95]. Moreover, PI3 kinase and AKT inhibitors of autophagy are upregulated in cancer. PTEN a tumor suppressor is also an activator of PI3 kinase and AKT pathway [91]. PINK1 (PTEN induced kinase-1) also has tumor suppressive activity [96]. PINK1 is essential cell cycle regulator. PINK1 is also an essential regulator of mitophagy.

Autophagy can be tumor promoting at the same time. Some of the chemotherapeutic agents such as DNA alkylating agent, antagonist tamoxifen, resveratrol, vitamin D3 and anthocyanins lead to accumulation of autophagosomes [97-100]. Similarly, hypoxia, hypothermia and radiation induce autophagy [91]. The induction of autophagy helps to combat the metabolic stress induced by chemo/radiation therapy [91]. This helps the cells to overcome apoptosis and promotes cell survival and thus tumor promotion. Moreover, core of the tumor where blood supply is insufficient, autophagy helps supply the nutrients and thus the survival. Thus, autophagy is a double edged sword in cancer.

2.3.2. NF-κB and IFNs: Linking inflammation, autophagy and cancer

Inflammation, autophagy and cancer are emerging trio that are interlinked to each other as discussed in chapter-1. In physiological condition, stress leads to the release of DAMPs in the cytoplasm as described earlier in the chapter. DAMPs then leads to the induction of inflammatory response and attracts macrophages at the site. This ideally leads to the tissue regeneration and repair; but over activation of macrophages leads to tumor promotion. Cell induces autophagy to combat the stress and diminishes the over activation of inflammatory response. This inhibits tumor promotion. Several conditions lead to defective autophagy such as alcohol, aging, obesity, chronic inflammation and chronic infection. These conditions lead to defective autophagy and thus lead to tumor promoting inflammation [13]. Inflammasome activation requires mounting of NALRP3 on mitochondria ultimately leading to IL1-β and IL-18 production [101]. This is depended on mitochondrial damage which induces mitochondrial ROS. Mitochondrial damage is

recognized by cell and undergoes Parkin mediated ubiquitylation. This further leads to recruitment of mitophagy adaptor protein p62 on mitochondria. This leads to elimination of damaged mitochondria and suppression of inflammation. p62 activation is regulated by NF-κB, an essential inflammatory mediator, that itself is responsible for IL-1β and IL-18 production [22]. This forms a self regulatory loop by which NF-κB regulates its own proinflammatory activity by removing damaged mitochondria [22, 102].

NF-κB and autophagy shares the same upstream signals and regulators and thus can control each other through positive or negative feedback loop. Autophagy leads to the degradation of IKKs, essential regulator of NF-κB signaling pathway [103, 104]. At the same time NF-κB leads to the upregulation of autophagy regulators Beclin-1 and thus induces autophagy [105]. p62 as discussed above is also activated by NF-κB. Type-I IFNs are also essential in linking autophagy and cancer. TANK-binding kinase 1 (TBK1), an essential regulator of IFN signaling are transiently recruited to the polyubiquitinated mitochondria, and the activated TBK1 phosphorylates p62 at S403. TBK1 inhibitor, BX795, prevents the p62-mediated autophagosomal engulfment of Parkin-recruited mitochondria [106]. Absence of autophagy results in the induction of ROS dependent RIG-1 signaling pathway that leads to the IFN induction [107]. Type-I IFNs treatment induces autophagy in certain human cancer cell lines such as HeLa S3, MDA-MB-231, T98G and A549 cell lines [108].

During antiviral signaling, MAVS and MITA are essential regulators of NF-κB and IFNs [17]. These proteins are located on ER-mitochondria interface. The degradation of mitochondria may down regulate the level of several mitochondrial and mitochondrial associated membrane resident proteins like MAVS, MITA regulating inflammation hence regulating inflammation in physiological limits. In fact, cGAS, an upstream regulator of MITA is observed to directly interact with Beclin-1 [109]. This inhibits cGAS mediated IFN production by enhancing autophagy mediated degradation of infectious agent. These proteins thus might be essential link between inflammation, cancer and autophagy. In the current study we have investigated the role of these proteins in connecting autophagy, inflammation and cancer.

2.4. MAVS and MITA: ER-mitochondria association and inflammation

Mitochondria is known to be central regulator of energy metabolism however the emerging evidences have clearly demonstrated the role in several nontraditional pathways like cell death, autophagy and inflammation. Recently the mitochondrial proteins have been identified that are central regulator of NF-κB and IFN pathway, suggesting the critical role in regulation of inflammatory pathways. The pathway is induced by RIG-I and TLR3 that recognizes the dsRNA a viral component as ligand. The amino-termini of RIGI and MDA5 contain two CARD domains that interact with the CARD domain of the mitochondrial adaptor MAVS (Mitochondrial adaptor of viral signaling). MAVS (also known as Cardif, VISA, and IPS-1), localized to the outer mitochondrial membrane contains an N-terminal CARD-like domain and a C-terminal transmembrane domain [15]. The C terminal 15 amino acid of MAVS targets to outer mitochondrial membrane and N-terminal CARD-domain interacts with the CARD of RIG-I and MDA5 [110]. An intact CARD domain and its localization to mitochondria both are necessary for the antiviral function of MAVS [15, 110-112]. MAVS is reported to interact with MFN1; after RLR activation, the complex MFN1–MAVS regulates either MAVS redistribution along mitochondria or the elongation of the mitochondrial network [113]. Elongation of the mitochondrial network enhances the association of MAVS with MITA [113]—an antiviral signalling adaptor localized in the endoplasmic reticulum membrane [16]—therefore amplifying the antiviral response. MAVS recruits TRAF3 and TRAF6 and further activates two cytosolic protein kinase complexes: the ’non-canonical’ IKK-related kinase TBK1 or IKKε associated with various adaptor proteins, such as TANK, NAP1 and NEMO; or one comprising IKKα, IKKβ and NEMO [114]. The TBK1 complex induces the phosphorylation and dimerization of the transcription factors IRF3 and IRF7, which translocate to the nucleus and bind to IFN-stimulated response elements (ISREs), thereby resulting in the expression of type I IFN genes and a set of IFN-inducible genes. The IKK complex activates NF-κB, subsequently promoting the expression of pro-inflammatory cytokines. Hence modulating this pathway to produce IFN in cancer cells to induce cell death can prove itself a very effective treatment.

Recent reports suggest the role of MITA independent of RIG-1 and MAVS signaling pathway. Evidences suggest MITA to be an essential part of DNA sensing pathway of

innate immunity. cGAS is a cytosolic DNA sensor which mediates IFN production by regulation of MITA. The stress induces the release of DAMPs inside the cytosol. Aberrant mitochondrial DNA packaging promotes escape of mtDNA into the cytosol [49]. cGAS senses such DAMPs and converts the cytosolic DNA into the cyclic GMP AMP structure (cGAMPs 2'-5') [50]. cGMAPs than activates MITA and subsequently IFN production. The reports suggest that this signaling molecule (cGAMP) is transferred to the neighboring cells via gap junctions and induces type-I IFN response in the nearby cells during viral infection [115]. Herpes viruses are reported to induce mitochondrial stress and mtDNA instability that triggers the antiviral priming response [50]. mtDNA can also be released in the cytoplasm because of other cellular stress which can be overcome by cells via autophagy. This release of mtDNA can also lead to IFN signaling inside the cell which can unnecessarily lead to cell death. To overcome this, cell keeps this pathway under check by means of apoptotic caspases such as caspase 3 and 7 [116, 117]. MAVS and MITA thus are essential regulators of IFN and NF-κB which lies in the center of connecting inflammation to cancer. They are in the center of regulating IFN and NF-κB; hence can be an important link to autophagy and cancer. The current study focuses on studying the role of MAVS and MITA in breast cancer in detail. As discussed inflammation, autophagy and breast cancer are emeging trio; hence the study will help understanding how these processes are interlinked to each other. The study will open new era of breast cancer therapeutics.