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The interconnection between the inflammasome and breast cancer

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Abstract Breast cancer is a condition of mammary cells that gain the capacity to abnormally proliferate and represents one of the cancers with an unpredictable evolution. Being a heterogeneous disease which present multiple subtypes, it remains an important problem for the biomedical domain. Inflammation influences the progression of the tumorigenic processes, being supported by the inflammasome complex and important signaling pathways which all associated promote breast tumor development. Oncogenic modifications modulate inflammatory microenvironment through inflammatory cells involvement, contributing to the development of more aggressive and challenging type of breast malignancy. Inflammasome is a cytosolic multiprotein complex composed by domain-containing protein (NOD)-like receptor, the apoptosis-associated speck-like protein containing a caspase activation and recruitment domain and caspase-1, involved in inflammation and pyroptosis. Over the years it has been proven that persistent inflammation associated with inflammasome activation promotes breast cancer initiation and progression and the purpose of this paper is to highlight the accumulated information on this subject and to understand its complexity.

Keywords: breast cancer, inflammation, inflammasome complex, pyroptosis

Introduction

The human breast represents an organized structure consisting of fatty tissue, lymph nodes, lobules, lobes and ducts, the last ones being coated by epithelial cells and myoepithelial cells which form a stratified epithelium sustained by a basement membrane (Chiorean *et al.*, 2013).

Cancer can be described by uncontrolled activities of cells due to DNA alterations or epigenetic factors (Provenzano *et al.*, 2018). The abnormal growth of mammary epithelial cells promotes the initiation of breast cancer (BC) but the progression is dependent on the ducto-lobular area and surrounding tissues affected. Due to this consideration, the histological classification of BC reveals two main types*: in situ* carcinoma and invasive carcinoma. Each type of breast carcinoma is sub-classified depend on the characteristics of the respective tumor, so *in situ* breast carcinoma can be described as lobular or ductal with five different subtypes (comedo, cribiform, micropapillary, papillary and solid). The other main type, invasive carcinoma is characterized by seven particular subtypes (tubular, ductal lobular, invasive lobular, infiltrating ductal, mucinous, medullary and papillary) (Ullah, 2019).

Another systematization of BC was developed based on molecular aspects, such as the expression profile of the estrogen receptor (ER), the progesterone receptor (PR) and the epidermal growth factor receptor 2 (HER2) (Provenzano *et al.*, 2018). Molecular subtypes identified over the years are Luminal A (ER or/ and PR positive, HER2 negative, protein Ki-67 negative), Luminal B (ER or/ and PR positive, HER2 positive, protein Ki-67 positive), HER2-enriched (ER or/ and PR negative, HER2 positive), Basal-like or triple negative (ER negative, PR negative, HER2 negative) (Ullah, 2019), Claudin low (ER negative, PR negative, HER2 negative, claudin 3, 4, 7 negative, E-cadherin negative) (Dias *et al.*, 2017), normal-like (ER or/ and PR positive, HER2 negative, low levels of protein Ki-67) and molecular apocrine (ER negative, androgene receptor positive) (Lehmann-Che *et al.*, 2011) (Fig. 1).

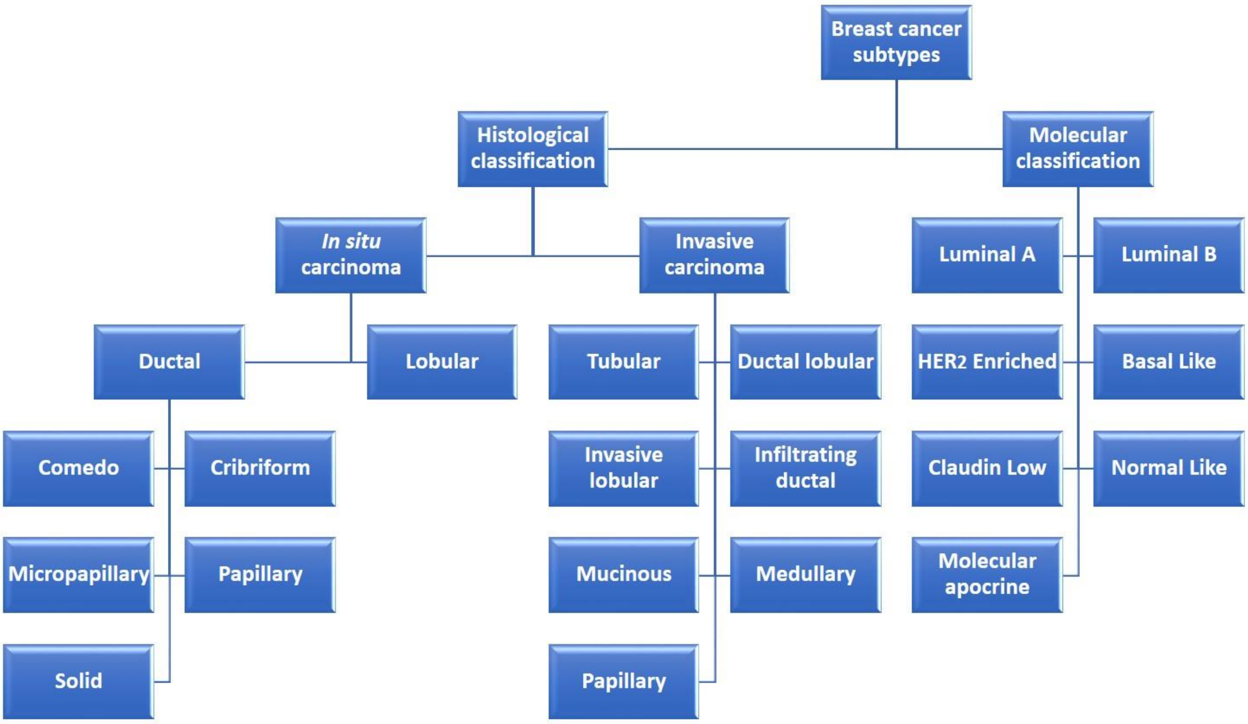

Fig 1. Breast cancer classification**.**

Also, it is speculated that initiation, progression and metastasis of some breast tumors are caused by cancer stem cells due to self-renewal capability, differentiation potential and similarities with normal mammary epithelial stem cells (Shipitsin and Polyak, 2008). Self-renewal potential is considered responsible for the increased number of genetic mutations in *GATA3, RUNX1, NCOR1, RB1, CDH1* and many others which are associated with different subtypes of BC (Anstine and Keri, 2019). Of course, there are other risk factors that can cause BC, such as family history, nullparity, menopause debut, oral contraceptive handling, abortion, hormone replacement therapy, radiation or lifestyle and nutrition habits (Buyukavcu *et al.*, 2016).

Considering the secretion and maturation of pro-inflammatory cytokines, the presence of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β and insulin resistance development, an interconnection between chronic inflammation and BC debut can be taken into consideration. Also, tumor initiation and propagation are determined by inflammatory response and some transcription factors, such as: nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), signal transducer and activator of transcription 3 (STAT3) and peroxisome proliferator-activated receptor (PPARα) (Zimta *et al.*, 2019).

Inflammasomes are cytoplasmatic multimolecular complexes compound of NOD-like receptors, pyrin member absent in melanoma 2 (AIM2) protein, the apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC) and caspase-1, which influence the progression of chronic inflammation through some key signaling pathways. The numerous research activities carried out in the field have suggested that many conditions, for instance cancer, autoinflammatory diseases and infections develop due to mutations in genes associated with inflammasome components. BC is influenced by the secretion of IL-1β, due to activation of inflammasome complex, which regulates angiogenesis and mediates the activity of adipocytes (Karki *et al.*, 2017).

The purpose of this paper is to understand the key events that realize the connection between inflammation, inflammasome complex and BC. This work contributes to the advancement of biomedical knowledge about BC by clear, concise and original illustration of the molecules and mechanisms involved in the progression of mammary carcinogenesis, supported by inflammatory processes or the inflammasome complex.

Inflammation

Inflammation is a non-specific mechanism of response and defense of the body to an aggression and also a pathological process that encompasses numerous phenomena (destructive, vasculo-exudative, proliferative and reparative) (Grivennikov *et al.*, 2010).

The histological feature of the inflammation is the leukocyte infiltrate. In the initial stages, the predominant cells are the neutrophils granulocytes. In the later stages and during the resolution of the inflammatory phenomenon, the mononuclear cells are the ones that predominate and which phagocytose and degrade in the phagocytic vacuole the etiological agent and necrotic remnants of the inflammatory process, releasing extracellular proteolytic enzymes, altering the inflamed area (Baumgarten and Frasor, 2012).

Cancer comprise numerous and heterogeneous cell types counting fibroblats, immune cells, adipocytes, endothelial cells. The connection between them is that they are capable of chemokines and cytokines secretion which influence cancer cells and tumor initiation, progression and metastasis (Coussens *et al.*, 2013).

Some carcinogens can induce somatic changes which lead to cancers development from "subthreshold neoplastic states" acknowledged as cancers initiation, characterized by DNA alteration. The second step named promotion is described by the cells vulnerability to irritants, just as phorbol esters, hormones, irritation or inflammation. This step is responsible for cell proliferation, recruitment of inflammatory cells and oxidative DNA damage due to presence of reactive oxygen species (ROS), with effects on the interaction with DNA (Yamanishi *et al.*, 2002).

Aberrant cells replication, proliferation and metastasis is supported by inflamed tissues, modifications of cell death and repair programs and altered inflammatory mechanisms (Coussens and Werb, 2002).

A credible assumption is that the infection and inflammation sites are culpable of malignancies arising. The connection between these three processes is orchestrated by signaling molecules, inflammatory cells and tumor cells, such as TNF-α. TNF-α mediates inflammatory cell populations and its function dysregulation can lead to aberrant processes and, sooner or later, to pathogenic processes (Moustakas *et al.*, 2002).

Inflammatory cells are capable of chemokines and cytokines' production, through which they control the growth, migration and differentiation of the cells from tumor microenvironment. In BC patients, increased cellular levels of inflammatory markers have effects on cell survival regardless of age or stage of tumorigenesis (Coussens and Werb, 2002).

An example that initiation and progression of BC is correlated with chronic inflammation is represented by up-regulation of the aromatase, an enzyme often found in breast tissue that is involved in estrogen biosynthesis. Up-regulation of aromatase is responsible for abnormal production of estrogen in breast tissue, which suggest that inflammation is correlated to BC through a common panel of biomarkers such as IL-1, IL-6, TNF-α and C-reactive protein (CRP).

Under specific prospects NF-kB activation can promote cell death. For example, the presence of doxorubicin, influence the tumor cell death through NF-kB down-regulation, but all these preliminary data require *in vivo* confirmation (Ivanov and Ronai, 2000). An alternative activation of NF-kB pathway is supposed to be involved in BC, this one is associated with TNF receptor-associated factor (TRAF3) and NF-kappa-B-inducing kinase (NIK) inactivation (Demicco *et al.*, 2005).

However, an increased level of TNF-α and IL-6 induced by the M1 type macrophages, connects chronic inflammation with mammary tumor progression and dysfunction of immune system. Both these processes combined support the spread of cancer (metastases) (De Boer *et al.*, 2017).

Most human neoplastic cells, influence the expression of chemokines in host cells, which normally regulates the migration of leukocytes during inflammation, but in neoplastic tissue their receptor system is modified for supporting tumor growth and progression (Mantovani *et al.*, 2001). Metastatic potential of malignant cells suppose the invasion and survival in ectopic tissue. A particularization in BC metastases is represented by the interaction between CXCR4 and the ligand SDF-1/ CXCL12, CXCL12 being highly expressed in BC metastatic sites, which generates chemotaxis of mammary tumor cells, mediating the process of metastases in cancer (Muller *et al.*, 2001).

The correlation between inflammation and inflammasome is direct, the inflammasome complex is responsible for caspase-1 activation, which influences the IL-1β and IL-18 activation. IL-1β and IL-18 are able to bind to their specific receptors and influence the NF-kB and MAPK pathways (Kasza, 2013).

a. NF-kB: involvement in inflammation and breast cancer

Transcription factor NF-kB, named „the master transcriptional switch in inflammation" is one of the main molecules involved in the development of innate immune and inflammatory responses. NF-kB modulates the activity of cytokines, molecules that influence the production of inflmmatory mediators, playing important functions in celullar processes, including in the initiation of carcinogenesis. A relevant exemple is the cellular homolog *c-rel* that encodes one of NF-kB subunit and share the same DNA binding domain with *v-rel* oncogene (Maeda and Omata, 2008).

NF-kB pathway is activated due to detection of some extracellular factors and phosphorylation and degradation of a kinase complex called IkB. IkB influences the dimerization of NF-kB and its liberation into the nucleus with the purpose of inflammatory process modulation and inflammasome mediated cleavage (Naugler and Karin, 2008).

A connection between inflammation and BC is given by NF-kB signaling pathway (Figure 2). It was proved that inhibition of NF-kB predisposes normal cells to apoptosis, because this transcription factor is important for cell survival, proliferation, migration and angiogenesis. NF-kB is a downstream regulator of some intracellular receptors of inflammation, for example Toll-like receptors (TLRs), considering its function in different cytokines expression levels and tumor cells survival (Bhatelia *et al.*, 2014).

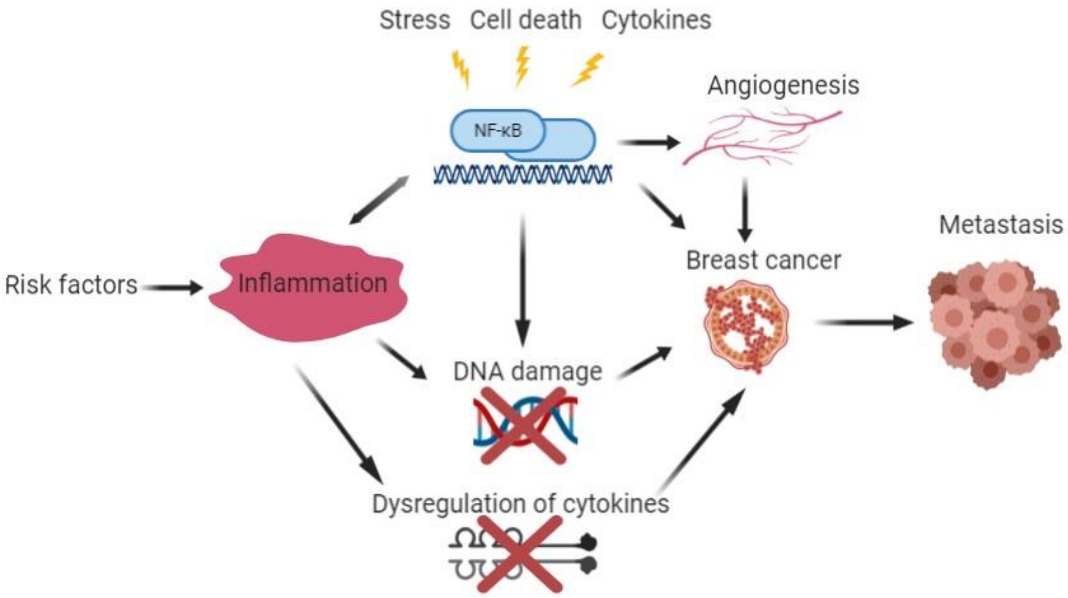

Fig 2. The connection between inflammation and breast cancer. Inflammation and NF-kβ signaling pathway are influenced by various factors. These processes, can lead to breast cancer initiation, progression and metastasis as a result of angiogenesis involvement, DNA damage and cytokines dysregulation.

The link between inflammation and BC is indicated by amplification and overexpression of nuclear factor kappa-B kinase (IKK) ε, involved in NF-kB signaling pathway. IKKε is translocated to the nucleus and summoylated after it was induced by DNA damage. The final step is phosphorylation and activation of NF-kB (Renner *et al.*, 2010).

b. ROS implication

The responsible organelle for oxidative stress is the mitochondria, which produce a noticeable volume of ROS due to persuaded defect in electron transport chain (Adam Vizi, 2005). ROS represent signaling molecules which contain in their molecular structure a specific number of oxygen atoms involved in organization of free radicals characterized by superoxide anion ($O^{\bullet 2-}$), hydroxyl radical ($HO^\bullet$), peroxyl radical ($ROO^\bullet$), hydroperoxyl radical ($HO^{\bullet 2}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O^3) and singlet oxygen ($_1O^2$) (Pavelescu, 2015).

In different circumstances, the origin of ROS production could be represented by the oxidative metabolism of mitochondria and as a response to diversified factors such as hypoxia, irradiation, bacteria, xenobiotics or cytokines (Grivennikova and Vinogradov, 2013).

Furthermore, apart from mitochondria, the peroxisomes and the cytochrome P450 system represent specialized sites for ROS generation, which contribute to BC promotion, initiation, progression and metastasis through genetic alterations and cellular damaging. The probability of carcinogenesis initiation is increased by the presence of products of lipid peroxidation within tumors, like malondialdehyde or 4-hydroxynonenal. All these products can lead to tumor suppressor gene dysregulation, process which propose the strong association between inflammation and carcinogenesis (Ahmad *et al.*, 2009).

Lu *et al.*, (2017), studied ROS implication in autophagy and apoptosis, observing the correlation between degradation of catalases and damage of nucleic acids, proteins or lipids, which turn into injure of various systems, cells death or p53 activation. Generation and accumulation of ROS aid the detachment of p53 from the Mdm2-p53 complex, leading to p53 activation through JNK signaling pathway. As a conclusion of the study, Lu *et al.*, declare that reduced generation of ROS was caused by silencing p53 gene. P53 is involved in numerous signaling pathways and mechanisms, such as cell cycle checkpoint regulation, genotoxic stress or ROS induced apoptosis.

Oxidative stress is a characteristic of tumor microenvironment. ROS produced in chronic inflammation are induced by activated neutrophils and macrophages which can modulate celullar DNA during cell division and activate DNA mutations due to repeated cellular disorganisation, loss and abnormal proliferation (Maeda and Omata, 2008).

ROS presence in inflammatory cells and epithelial cells are culpable for the disturbance of biomolecules such as nucleic acids, proteins or lipids. ROS have a key role in cellular damage, necrosis, genomic instability, mutagenic lesions and inflammation related carcinogenesis, being also involved in immunogenic and inflammatory cell death or pyroptosis (Kawanishi *et al.*, 2017).

Manifestation of inflammation are also displayed by solid tumors which attract tumor associated macrophages (TAMs), innate immune cells associated with cytokines like IL-6. TAMs are involved in tumor cells growth, invasion and metastsis (Triner and Shah, 2016). Increased number of tumor cells or death cells may indicate the presence of inflammation or tissue necrosis (Germolec *et al.*, 2009).

Interrelationship between ROS and different signaling pathways has been experimentally demonstrated. In NF-kB signaling pathway, ROS inhibit the phosphorylation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IkBα) by IKK with the activation of NF-kB signaling pathway (Reynaert *et al.*, 2006). In the same time, ROS regulate a redox-sensitive kinase upstream of IKK, called mitogen-activated protein kinase kinase kinase 1 (MEKK1) and activate the NIK (Kim *et al.*, 2008).

Inflammasome

A critical molecular assembly leading to inflammation is inflammasome. Inflammasome is a cytoplasmatic protein complex responsible for cystene protease caspase-1 activation and which recognizes the pathogen and intervenes in host defense. The concept of inflammasome includes sensors, adaptors and enzymes. The presence of specific stimuli and their detection, nucleates the adaptor protein apoptosis-associated speck-like protein containing card (ASC) to form „speck” or foci and to interact with caspase-1, which produce the active subunits p10 and p20. Consequent to activation of the two subunits, the inflammatory cell death or pyroptosis is induced through cytokines IL-1β and IL-18 (Sharma and Kanneganti, 2016). All these components of inflammasome complex form a wheel shaped architecture related to that formed by the apoptosome complex, realizing the correlation between their similar functions (Broz and Dixit, 2016).

a. Systematization and assembling

The inflammasome assembling is managed by three elements, platform, adaptor and effector proteins. The platform proteins are represented by NOD-like receptor (NLR) family which is classified into three subcategories, NODs and Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing (NLRPs). The adaptor protein is useful for recruitment of pro-caspase-1 and intervenes in the elaboration of inflammatory responses. NLRP3 and NLRP6 are two types of inflammasomes which do not have caspase-activation and recruitment domains (CARD) (Lamkanfi and Dixit, 2012).

Some studies theorize that endoplasmic reticulum and mitochondria interface represent a platform for inflammasome assembly. The receptors detect endogenous damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) and determine the oligomerization of inflammasome, caspase-1 activation and IL-1β secretion and maturation (Bhatelia *et al.*, 2014).

The mechanism of caspase-1 activation is completed by the inflammasome complex. The ASC protein is recruited and interacts with NLR or AIM2 domains, contributing to the recruitment of pro-caspase-1. Further on, activated caspase-1 is involved in pro-IL-1β and pro-IL-18 maturation, pores formation, elaboration of inflammatory answers and pyroptosis (Gassart and Martinon, 2015).

There are two forms of inflammasome activation. The canonical inflamasomme activation consider two important steps, the first one is relying on NF-κB signaling pathway activation through recognition of DAMPs and PAMPs by TLRs; the second step presume recognition of DAMPs and PAMPs, molecules released from damaged cells, by NLRs (Kolb *et al.*, 2016).

The non-canonical activation of inflammasome is realised by caspase-8 or caspase-11, CARD 9, Malt1, Bcl-10 and ASC, which can induce pyroptosis (Gringhuis *et al.*, 2012). Due to ethical issues, the most studied is caspase-11 which interacts with lipopolysaccharides, promotes the activation of caspase-1 and detachment of gasdermin (GSDMD) domains, and induces pyroptosis (Kayagaki *et al.*, 2015).

The first step in inflammasome assembling is recruitment of ASC by pyrin domains (PYD) interactions and recruitment of caspse-1 by CARD interactions. This intercommunications lead to ASC specks formation and their release into extracellular medium to contribute at inflammatory responses development (Franklin *et al.*, 2014). ASC specks together with PYD form filaments with a long helically structure and interact with CARD, stimulating the recruitment and activation of caspase-1, IL-1β, GSDMD-D and pyroptosis promotion (Dick *et al.*, 2016)

b. NLRP3

Numerous studies were conducted for the evaluation of NLRP3, with three plausible interpretations of its activation mechanisms. The first one is based on nucleotide-binding and oligomerization domain (NACHT) and leucine-rich repeat (LRR) interaction, which results in auto-suppression of NLRP3. But in presence of DAMPs and PAMPs, the auto-suppression is inverted, influencing the conformational modifications and mechanisms which involve the caspases family (Man and Kanneganti, 2015). Another hypotheses depend on the presence of channels and pore which determine the activation of NLRP3 through extracellular ATP molecules. ATP bind to P2X purinoceptor 7 (P2X7), inducing a potassium (K+) efflux, allowing DAMPs and PAMPs to penetrate the cell, K+ efflux being considerate a downstream mechanism for NLRP3 activation. Also, is considering that activation of NLRP3 is influenced by the amount of ROS produced, but only ROS cannot determine the entire activation mechanism (Abais *et al.*, 2015).

A study realized by Bauernfeind *et al.*, (2009) proposes an activation mechanism of the inflammasome in macrophages based on two signals. The first one or priming signal depends on TLRs and TNF-α receptor that intervene in the NF-kB signaling pathway leading to post-translational alteration of NLRP3. The second signal is involved in IL-1β and IL-18 discharge mediated by caspase-1 and initiation of pyroptosis.

On the other hand, caspase-1 activity stimulates IL-1β and IL-18 secretion, promotes inflammation and pyroptosis, after inflammasome activation, the substrate for IL-18 being produced (Lehmann-Che *et al.*, 2011) (Fig. 3). NLRC4 inflammasome activation stimulates IL-1β production and regulates the expression of adipocyte-mediated vascular endothelial growth factor A and angiogenesis, and as a result, BC progression is increased (Kolb *et al.*, 2016). A study from 2007 suggested that expression of IL-1β is higher in invasive ductal carcinoma than in *in situ* ductal carcinoma and it is correlated with a threatening phenotype (Chavey *et al.*, 2007).

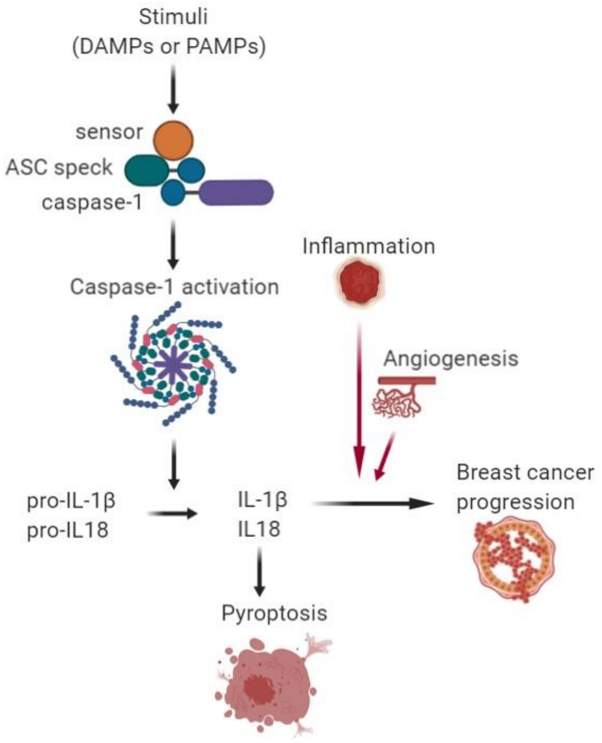

Fig. 3. The inflammasome mechanism in breast cancer progression. Recognition of stimuli (DAMPs and PAMPs) by sensors specific to inflammasome complex, promote inflammasome activation and pyroptosis initiation. The inflammasome promotes breast tumor progression sustained by IL-1β and IL-18 induced inflammation and angiogenesis.

Recent studies, suggest that inflammation induced by inflammasome complex or IL-1 signaling pathway support BC initiation and progression. Tumorigenesis is supported by adaptor protein ASC which has numerous functions in this regard. A properly example is that viability and proliferation of tumor cells are increased when the gene encoding ASC is knockdown (Liu *et al.*, 2016).

Guo *et al.*, (2016) reported that inflammasome activation influences the infiltration of TAMs and myeloid-derived suppressor cells. IL-1R signaling pathway can be inhibited, using an IL-1R antagonist or anti- IL-1R antibody, which determine the inhibition of tumor growth and suppression of myeloid cells. The conclusions of the study were that an inflammatory microenvironment is promising for BC initiation and progression and that the IL-1 signaling pathway inhibition can represent a possible therapeutic target.

Kolb *et al.*, (2016) identified obesity as a principle risk factor for BC initiation associated with NLRC4 inflammasome. They show that obesity represents a good platform for myeloid cells increasing levels together with IL-1β production, which also promotes angiogenesis. Making a correlation between this study and the one employed by Weichand *et al.*, (2017), in BC, NLRP3 inflammasome activation and IL-1β production can be induced as well by S1P receptor 1.

NLRP3 inflammasome is also involved in leptin induced BC, because leptin plays a decisive role in NLRP3 inflammasome activation through estrogen receptor and ROS production. The concluding events are represented by apoptosis and cell cycle promotion (Raut *et al.*, 2019).

Pyroptosis

Over the years, numerous studies have revealed different types of cell death, such as apoptosis, necrosis, autophagy, necroptosis, anoikis and pyroptosis (Shi *et al.*, 2017). The word pyroptosis is borrowed from the Greek language, being compound from suffix “pyro” which is translated fire and “ptosis” adapted for inflammatory cell death (Cookson and Brennan, 2001).

Pyroptosis depends on enzymatic capacity of caspases family (caspase-1/ 4/ 5/ 11) to perform inflammatory actions. Activation of inflammasome complex through canonical or non-canonical way will lead to caspases family activation and in the end to cell swelling, cell lysis and pyroptosis. Another way to promote and initiate pyroptosis is completed by GSDMD family, principally by N-terminal domain of gasdermin D (GSDMD D), which is recruited to the plasma membrane. The N-terminal domains are involved in pores assembling from 16 protomers, which change integrity of the cell membrane. A sign of pyroptosis debut is represented by membrane permeabilization by cause of pores resulted after caspase-1 activity (Ding *et al.*, 2016).

In embryonic development and under homeostatic conditions pyroptosis is absent, being mostly activated in pathophysiological conditions, such as inflammatory and autoinflammatory diseases. Pyroptosis is able to influence initiation, proliferation, invasion of BC and metastasis (Walle and Lamkanfi, 2016).

a. Pyroptosis and inflammasome

Pyroptosis is often oberved in macrophages, neutrophils, dendritic cells, T cells, endothelial cells or epithelial cells which express superior levels of caspses. Caspase-1 is not active in cytosolic medium, it is activated after the recruitment to inflammasome without or with adaptor protein ASC.

Activation of patern-recognition receptors (PRR) from inflammasome complex structure can activate inflammatory responses and in the end to turn on pyroptosis. PPR recognize molecules such as DAMPs or PAMPs, leading to the assembling of ASC speck and afterwards links NLR to caspase-1. Activation of caspase-1 stimulates pyroptosis initiation through canonical activation pathway of inflammasome, but, on the other hand, the non-canonical activation of inflammasome is stimulated by activation of caspase-11 and caspase-4/ 5 (Kayagaki *et al.*, 2015).

Caspase-1 activation leads to production and maturation of IL-1β and IL-18. A possible explanation for IL-1β and IL-18 discharge into the extracellular medium can be the activation of pyroptosis, which manages the inflammatory factors release, such as nuclear protein high mobility group box 1 (HMGB1), S100 proteins or IL-1α (Walle and Lamkanfi, 2016).

GSDMD is a substrate for caspases family, which can be separated into specifically domains by caspase-1, 4, 5, 11 (Shi *et al.*, 2015). Some studies suggest that GSDMD is involved in the pores formation process, in the intracellular components discharge (Liu *et al.*, 2016) and IL-1β secretion, but not in its maturation (Ye *et al.*, 2018).

b. Pyroptosis involvement in cancer

Pyroptosis is a caspase-1-dependent cell death, which consists of inflammatory response, cellular lysis and release of intracellular components due to cell membrane damage. Due to inflammasome activity, pyroptosis has some common charasteristics with apoptosis and necrosis, such as staining of anexin V, maturation of caspases, nuclear condensation, DNA fragmentation, the appearance of pores formed into the membrane, osmotic lysis and intacellular components discharge (Lamkanfi *et al.*, 2008; Sharma and Kanneganti, 2016). Some particularities of pyroptosis have been observed, such as the disruption of cell membrane integrity and absence of nuclear fragmentation. A final step in development of pyroptosis is represented by the pores formation in the membrane (Bergsbaken and Cookson, 2007).

Pyroptosis is determined by GSDMD D activation consequent to cleavage by caspase-1, being also a substrate for this pro-inflammatory enzyme. GSDMD D activation is also dependent on membrane degradation (Karki *et al.*, 2017).

c. Interrelationship between inflammasome components and breast cancer

Inflammasome complex is involved in the modulation of viability and proliferation of tumor cells. A study realized by Pham *et al.* (Pham *et al.*, 2020), suggested that BC is promoted through inhibition of apoptosis and cell cycle progression. In their study, the researchers observed the activity of three inflammasome inhibitors (MCC950, IL-1RA, globular adiponectin), which influence different steps of inflammasome activation. Inhibition of NLRP3 inflammasome or caspase-1 determines inhibition of MCF-7 and T47D BC cells growth. These results suggest that inflammasome activity promotes tumor development. In this study, the activity of inflammasome inhibitors could not be observed on triple-negative MDA-MB-231 BC cells, indicating the probability that ERα signaling pathway is involved in the promotion of breast cancer by inflammasome activation.

Before this study, Urra *et al.* (Urra *et al.*, 2016) demonstrated that in breast tumor tissue, the ER stress marker genes are overexpressed. So, ER stress contributes to breast tumor cells proliferation through apoptosis and cell cycle arrest modulation. Also, abnormal secretion of adipokines, such as leptin, influences BC progression through NLRP3 inflammasomes activation (Raut *et al.*, 2019).

On the other hand, the development of triple negative breast cancer (TNBC) is supported by IL-1 family overexpression, which influences the tumor cells proliferation, BC progression and metastasis (Holen *et al.*, 2016). Tumor microenvironment is supported by IL-1β and IL-18 pro-inflammatory cytokines. High levels of IL-1β and IL-18 were detected in BC patients, representing a characteristic of a poor prognosis (Perrier *et al.*, 2009). IL-1β influences tumor cells proliferation, initiation and progression of carcinogenesis, metastasis and host-tumor interactions (Apte *et al.*, 2006).

Another factor which is responsible for the connection between cancer and inflammation, is represented by cancer-associated fibroblasts (CAFs). CAFs are involved in recruitment of immune cells, cancer initiation and progression, angiogenesis and remodelation of the extracellular matrix (ECM) (Gascard and Tlsty, 2016). In 2019, Ershaid *et al.* (Ershaid *et al.*, 2019), observed that CAFs are involved in upregulation of NLRP3 inflammasome during BC progression. The results of the study, indicated that during BC, some genes involved in NLRP3 inflammasome activity are upregulated in CAFs (*p2rx7, nlrp3, casp1, IL1a, IL1b*).

Bruchard *et al.* (Bruchard *et al.*, 2013), studied the characteristics of 4T1 mammary adenocarcinoma induced on mice. The treatment applied to mice provoked NLRP3 inflammasome activation, IL-1β and IL-17 secretion by CD4+ T cells. All of this concluded in reduced efficacy of chemotherapy.

Kolb *et al.* (Kolb *et al.*, 2016), observed that the expression of NLRC4 analyzed from breast tumor tissue samples was increased. Thus, BC initiation and progression is sustained by NLRC4 inflammasome activation and obesity.

d. Inflammation, inflammasome and breast cancer

There are two models in which the connection between inflammation, inflammasome and breast cancer can be achieved, an extrinsec pathway known as inflammation-induced carcinogenesis and an intrinsec pathway specified in literature as cancer-associated inflammation (Kundu and Surh, 2012). After cancer initiation, the intrinsec pathway will be activated by tumor cells, performing the intersection between both pathways with the purpose of cancer progression and metastasis (Grivennikov *et al.*, 2010).

After inflammasome activation, IL-1β and IL-18 will be released and will intervene in the initiation of inflammation and alteration of DNA repair systems (Lin and Zhang, 2017).

DNA damage is amplified by inflammatory cytokines which influence the ROS and reactive nitrogen species (RNS) production in cells. In addition, high oxidative stress can affect numerous molecules, such as proteins, lipids, nucleic acids and intermediate metabolic products and determine DNA repair systems interruption and initiation and progression of carcinogenesis (Kundu and Surh, 2012).

A study realized by Pizato *et al.* (2018) investigated the anti-cancer effect of an omega-3 fatty acid, docosahexaenoic acid (DHA) exercised over MDA-MB 231 breast cancer cell line. The results shown that DHA induced pyoptosis in breast cancer cells through activation of caspase-1, cleavage of GSDMD domains, secretion and maturation of IL-1β and IL-18 and pores formation into the membrane. Besides, the DHA presence is influencing the HMGB1 transposition from nucleus to cytoplasm as a consequence of caspase-1 activation. They concluded that pyroptosis is an essential process for host defense along pathogens which can promote infected cells expulsion.

Therapeutic anti-cancer strategies

An anti-tumor effect can also be induced by cytokines and chemokines. The inflammasome activity can be connected with tumor mechanisms through its involvement in cytokine and chemokine secretion. Some research activities showed that in the absence of NLRP3 inflammasome activation, the tumor progression and metastasis invasion were less aggressive (Karan, 2018). Interesting results were obtained by Chow *et al.* in breast cancer model in which the activity of NLRP3 inflammasome activation and caspase-1 activity were inhibited. They observed that tumor growth and metastasis were delayed and the infiltration of myeloid derived suppressor cells (MDSCs) was decreased. Also, inhibition of NLRP3 and secretion of CCL5 and CXCL9 chemokines, influences the infiltration and anti-metastatic effect of natural killer (NK) cells (Chow *et al.*, 2012). Besides, NLRP3-deficiency is associated with immunogenic reprogramming in the presence of tumor cells (Daley *et al.*, 2017).

Some studies (Kopalli *et al.*, 2018; Shao *et al.*, 2015), suggested a couple of inhibitors which are responsible for NLRP3 inflammasome inhibition, reduced secretion of IL-1β and reduced tumor growth. An example of an inhibitor is withaferin-A, with effects on the secretion of specific cytokines and chemokines and which affects the architecture of inflammasome complex (Dubey *et al.*, 2018). Another inhibitor is thalidomide, which inhibits the activity of NLRP3 and affects the production and maturation of IL-1β. Its action is based on inhibiting caspase-1 activity and interrupts the IL-1β and IL-6 association (Keller *et al.*, 2009).

NLRP3 inflammasome activation can be determined by ATP recognition by P2X7 receptor, which is an indicator of chemo-resistance (Vincent *et al.*, 2010). Considering that in the NLRP3-caspase-1 cascade an important function is performed by P2X7 receptor, its inhibition can determine decrease inflammation (Adinolfi *et al.*, 2012). López-Castejón and later, Ludwig-Portugall, observed a molecule named cytokine release inhibitory drug 3 (CRID3, CP-456,773) with effects on ASC protein oligomerization, being a possible target for cancer therapy (López-Castejón *et al.*, 2012; Ludwig-Portugall *et al.*, 2016). Some antagonists of IL-1β are represented by IL-1RA anakinra which inhibits some markers of cancer initiation and progression (Wu *et al.*, 2018) and canakinumab (ilaris) which specific neutralizes IL-1β (Isambert *et al.*, 2018).

Andrographolide is a labdane diterpenoid which can affect the tumor cell invasion, decreases cell proliferation and cause cell death, through NF-kB signaling pathway (Zhang *et al.*, 2014). Experiments performed on breast cancer models showed that andrographolide inhibits NF-kB signaling pathway, suppresses osteolysis and cyclins and cyclin-dependent kinases, activates p53 protein and increases apoptosis (Zhai *et al.*, 2015). Andrographolide has negative effects on tumor cells survival, proliferation and angiogenesis (Shi *et al.*, 2008).

In 2008, Lin and his team investigated the influence of cresol tested as a treatment for breast cancer. The results indicated that the activation of NLRP3 inflammasome was inhibited and secretion of IL-1β was significantly reduced (Lin *et al.*, 2008). Apo-9-fucoxanthinone was also tested on two breast cancer cell lines, MCF-7 and MDA-MB-231. The conclusions were that apo-9-fucoxanthinone determined the inhibition of NLRP3 inflammasome, reduced breast tumor cells' proliferation and promoted cells' death (Moussavou *et al.*, 2014).

Conclusion

Proliferation, invasion and metastasis of BC are sustained by inflammation through various transcription factors and promoted by TAMs, tumor cells, adipocytes and fibroblasts, which produce pro-inflammatory cytokines. Together with various risk factors which provide an assuring inflammatory microenvironment for development of this disease, inflammasome activation induced by inflammation sustain tumor initiation and progression.

The transcription factor NF-kB, is a moderator in elaboration of inflammatory responses, being involved in carcinogenesis initiation and evolution. Changes in NF-kB signaling pathway, such as amplification and overexpression of IKKε, can determine DNA damage and lead to cell death.

The correlation between inflammation and inflammasome complex is realized by NF-kB signaling pathway. TLRs and TNF-α receptors interfere in this pathway and lead to the emergence of the activation signal represented by production of IL-1β and IL-18, mediated by caspase-1 activity. In turn, activated caspase-1 stimulates proteolytic cleavage, encouraging inflammation and pyroptosis or inflammatory cell death, after inflammasome activation.

Inflammasome assembly is provided by platforms (endoplasmic reticulum and mitochondria), which allow activation of caspase-1, stimulate inflammatory cytokines' activity and ultimately initiation of pyroptosis.

In order to understand the connection between inflammation, inflammasome complex and BC, the key molecular events that link these processes are exposed in this work. BC initiation and progression, supported by inflammation and inflammasome complex, must be studied in detail, considering that through caspase-1 and inflammasome sensors, different signaling pathways can be regulated. A future direction in this field can be represented by development of anticancer therapy based on anti-inflammatory treatments in combination with other anti-cancer agents.

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