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Main cell signalling pathways involved in breast cancer

1. Introduction

Breast cancer (BC) is a type of cancer that targets cells of the breast. Most types of breast cancer have an epithelial origin, and therefore, they are categorized as carcinomas. Cancer cells start to spread when they enter the lymph system and the blood to reach other areas of the body. Once they have invaded the lymph vessels, cancer cells attack the lymph nodes and the possibility of developing metastases is much higher (American cancer society, 2019).

Breast cancer can grow in several areas of the breast. Thereby, depending on where cancer has started, it can be non-invasive (in situ) or invasive breast cancer. The non-invasive is located only in the ducts of the breast. Invasive cancer is the most common type, and it develops when cancer has spread to the surrounding breast tissue (NHS, 2019).

In the UK, breast cancer is the most common cancer, affecting 1 in 7 women during their life. The likelihood that cancer will develop in the next 10 years increases with age, and therefore women over 50 are much more vulnerable than other age groups (Desantis et al., 2013; Cancer research UK, 2021). Between 20% and 30% of cases turn into metastatic cancers, and 90% of these types of cancer are deadly (Nola et al., 2012). For this reason, it is very important to find an efficient way to cure this disease.

Several treatments are put in place to help the body fight against breast cancer. Chemotherapy, monoclonal antibody therapy, radiotherapy, surgery, hormonal therapy, and immunotherapy contribute to stop the spreading and eradication of this disease. These treatments target the main cell signalling pathways involved in the development of breast cancer to cease the disease growth.

2. Signalling pathways

Numerous elements influence the division of breast cancer stem cells (BCSC). Ion channels and cell membrane receptors react with elements such as antibodies, hormones, and cytokines from the outside of the cell. As a result, cell signalling pathways can be triggered (Kamaruzman et al., 2019).

The cell proliferation process consists of the division of a cell, and it is regulated by growth factor receptors (GFRs). Cancer cells have specific surface markers, and they play a key role in the identification and eradication of the disease (Palomeras et al., 2018). The main GFRs in various cellular functions are the epidermal growth factor receptor (EGFR) and the Human

epidermal growth factor receptor 2 (HER2). They are part of the receptor tyrosine kinase family, and their overexpression is associated with a high death rate due to breast cancer (Kamaruzman et al., 2019). EGFR is made of four sub-proteins, whereas HER2 is an oncoprotein, involved in apoptosis prevention and proliferation, causing breast cancer progression (Zhang et al., 2017).

The main pathways involved in breast cancer are Notch, Hedgehog, and Wnt/β-catenin signalling pathways. Mitogen-Activated Protein Kinase (MAPK), PI3K/AKT, and Janus kinase/signal transducer and activator of transcription (JAK/STAT) are other important signalling pathways. All these will be reviewed individually in the next sections.

2.1. Notch signalling pathway

This pathway focuses on the interaction between one of four Notch receptors on one cell and the ligand DSL (Delta/Serrate/LAG-2). The DSL ligands are transmembrane proteins, and five different types are known: Jagged1, Jagged2, Delta-like1, Delta-like3, and Delta-like4 (Kamaruzman et al., 2019).

This communication causes proteolysis of the Notch protein, inducing several signalling cascades reactions. This implicates complexes of proteins that activate the Notch target genes. These genes are associated with tumour development. MiR-146a can trigger the development of breast cancer stem cells along the process. Several proteins can suppress or promote this type of pathway (Acar et al., 2016).

In breast cancer, the receptors and their ligands are overexpressed. Notch is associated with cellular activities such as apoptosis, epithelial to mesenchymal transition (EMT), hypoxia, and metastasis (Song & Farzaneh, 2021). This pathway plays an important role in radio resistance and chemoresistance (Palomeras et al., 2018).

2.2. Hedgehog signalling pathway

This type of pathway influences cell regeneration, differentiation, proliferation, maintenance, survival, tissue homeostasis, and tumour initiation. Hedgehog signalling interacts with pro-oncogenic factors and other signalling pathways. As an example, it is linked to the notch pathway using the connection between the EGFR pathway and the DSL ligand Jagged 2 (Song & Farzaneh, 2021).

The hedgehog signalling pathway starts with the Hedgehog ligand binding to its correspondent receptor, Ptch 1. Consequently, the transduction signal is not inhibited, and the protein Smoothened, the seventh transmembrane protein, is activated. Lastly, factors such as GLI 1, 2, and 3 are stimulated, and a zinc finger transcription factor activates the hedgehog target genes. These genes include Bcl-2, Ccnd1, and Myc family genes (Kasper et al., 2009).

The aberrant activation of GLI factors is an indicator of breast cancer cells' presence. GLI2 and GLI1 are needed for BC cell lines growth (Kasper et al., 2009). GLI1 is associated with α6β1 integrin, which triggers focal adhesion kinase signalling (FAK) and influences the self-renewal capacity of cancerous cells (Song & Farzaneh, 2021).

2.3 Wnt signalling pathway

Breast cancer is the only type of cancer involved in the Wnt signalling pathway. It affects the phenotype shaping of breast cancer, the metastasis, proliferation process, stemness maintenance, and immune microenvironment regulation. There are three types of Wnt signalling and each of them has Wnt proteins (WntX) that bind to different receptors. Consequently, various downstream pathways are initiated. All noncanonical and canonical Wnt signalling pathways target CD44, regulating the stemness of breast cancer cells (Xu et al., 2020).

The main one is the canonical Wnt pathway, which is dependent on β-Catenin. Several Wnt ligands bind to frizzleds (Fzds), primary receptors formed by 7-transmembrane proteins. The lymphoid enhancer factor (LEF) and the T cell factor (TCF) are involved in this pathway, causing cell proliferation and stemness maintenance (Song & Farzaneh, 2021).

Secondly, the Wnt-PCP signalling pathway uses different Wnt proteins from the canonical pathway and they target various receptors on the proximal side, such as Vangl2, Celsr1, Prickle, Intu, and Dvl; and receptors on the distal side, like Invs and Fzds. All these transmembrane complexes activate transducers, inducing several signalling cascades reactions. This leads to the formation of lateral asymmetry in epithelial structures, cell migration, and polarity that affects cellular adhesions and the cytoskeleton (Xu et al., 2020).

Lastly, the Wnt Ca+ signalling pathway plays an important role in early embryogenesis, inflammatory response, interneural communication, and cancer progression. It starts with the binding of the protein Wnt5 and the receptor Fzd2. After the activation of molecules like PLC and $InsP_3$, protein kinase C (PKC) is triggered and cell-division cycle 42 (Cdc42) is stimulated. $InsP_3$ opens calcium channels when binding to receptors $InsP_3Rs$. An increase in Ca^{2+} levels activate several calcium-dependent kinases, putting the nuclear factor of activated T cells (NFAT) in action (Xu et al., 2020).

2.4 Mitogen-activated protein kinase (MAPK) pathway

Six types of MAPKs play a role in amplifying and delivering extracellular signals. They take part in the ERK pathway, which integrates external signals into signalling factors to promote proliferation and cell growth from the presence of epidermal growth factor (EGF). This pathway is activated by the binding of ligand and receptor tyrosine kinase (RTK). Consequently, the Ras protein is stimulated and signals affecting gene expression and transcription are transmitted to the nucleus. The overexpression of mitogen-activated

proteins is found in breast cancer cells, and it has been associated with the metastatic potential of this cancer.

2.5 PI3K/AKT signalling pathway

Protein kinase B, also known as AKT, regulates cell motility, glycogen metabolism, cell survival, and proliferation. The PI3K/AKT signalling pathway starts when growth factors such as EGF bind to their tyrosine kinase receptor. The protein PI3K is activated, and Protein kinase B is phosphorylated, targeting then rapamycin (mTOR) to stimulate cell growth. The catalytic action of AKT influences more than one hundred substrates that regulate processes such as metabolism, apoptosis, proliferation, protein expression, and breast cancer metastasis (Hinz & Jücker, 2019; Baselga, 2011).

PI3K and AKT are overexpressed in 38% of breast cancer cases. Some mutations are shown in PIK3CA, and only one amino acid is substituted. These changes occur in up to one-quarter of the cases in this disease, and they enhance enzymatic function and downstream signalling factors to stimulate oncogenic transformation (Kamaruzman et al., 2019).

2.6 JAK/STAT Signalling Pathway

Cytokines and other extracellular stimuli start the JAK/STAT pathway. Signal transducer and activator of transcription (STAT) and Janus kinase (JAK) are the intracellular proteins that bind to the transmembrane receptor to send signals to the nucleus and modify gene expression and DNA transcriptions. This pathway influences haematopoiesis, stem cell maintenance and takes part in the inflammatory response. It is also involved in cell differentiation, proliferation and participates in the regulation of cellular apoptosis (Kamaruzman et al., 2019). It has been observed that the overexpression of STAT3 is present in 69.2% of breast cancer tumours (Dolled-Filhart et al., 2003).

3 Conclusion

Breast cancer is an important disease that affects women and a minority of men all around the world. In each signalling pathway, the binding between the corresponding protein to its receptor has a big influence on the regulation of cell proliferation and cancer growth. Studying the signalling pathways involved in breast cancer is a good approach to help fight and cure this disease.

Body differentiation self from nonself

1. Introduction

The recognition of pathogens and nonself material is crucial to protecting the human body. This is a very important property of the immune system; otherwise, self-cells would be destroyed. Identification of self and nonself cells represents a big challenge when accepting and rejecting blood transfusion and organ transplantation.

2. Identification

Pathogens have associated molecular patterns on their surface, known as pathogen-associated molecular patterns (PAMPS). These include mannans, a type of sugar associated with bacteria, and lipopolysaccharide and lipoteichoic acid associated with bacteria. These are recognized by pattern recognition receptors (PRR) on the host cell (Horrocks, 2021).

Another type of identification method is the soluble factors within the host which can bind to features of microbial surfaces, such as mannose-binding lectin, C-reactive proteins, and MHC. Then the receptors of the host cells will recognize the bound molecules and the immune system will attack the pathogen. As an example, opsins are molecules that can bind to the surface of the pathogen and after they have been identified by phagocytes, phagocytosis is promoted (Horrocks, 2021).

An antigen is any molecule that activates the immune system response. It can be a molecule of glycolipid, protein, polysaccharide, or glycoprotein on the cell surface membrane. The recognition of the antigen activates B cells to produce antibodies, which act as an opsin. Antigens and antibodies form the antigen-antibody complex when they bind, and phagocytes will target those complexes to start phagocytosis (BioTopics, no date).

The cell membrane contains glycoproteins which take part in the cell identification system. This is the family of MHC molecules, which can also be called the human leukocyte-associated (HLA) antigens. They allow the body cells to recognize self-cell and nonself-cells by identifying the correct glycoproteins sticking out of the cell surface. MHC contains complex carbohydrates linked to them and acts as a cell's identity badge. The cells of the immune system will attack any cell without the correct glycoproteins sticking out of it because they have recognized it doesn’t belong (Bledsoe, 2011). MHC molecules take part in the binding of peptide fragments of proteins that have been synthesized, class I molecule within the cell, and class II have been proteolytically processed and ingested by the cell (Chaplin, 2010).

3. Immune attack

After nonself material has been recognized, the first response is from the innate immune system. The phagocytes recognize the PAMPS and ingest, digest, and destroy the foreign material. Others features in the bloodstream are also able to attack such as proteins of

the complement system and antimicrobial peptides. As a result of the intrusion, elements involved in the innate system produce inflammation. Vasoactive and chemotactic factors trigger a local increase in capillary permeability and blood flow. As a result of increased vascular permeability and vasodilatation, permeable capillaries allow an influx of fluid and cells. Macrophages go through walls of capillaries and release cytokines to recruit further cells and continue with the immune response. This attracts phagocytes such as neutrophils and other leukocytes to the site of inflammation and destroys the pathogen (Horrocks, 2021).

The adaptive response usually takes control of the immune system response after several days, and after the nonself material had passed through the innate immune defences. This response consists of a humoral and cellular branch. B cells and T cells works together for the activation and production of antibodies. The macrophages, dendritic cells, and B cells contain MHC molecules on their cell membrane surface, and these can bind to the antigen of the pathogen. Two types of T lymphocytes are part of the acquired immune system, T helper T-cells (T_H) and T cytotoxic or cytolytic T-cells (T_c or CTL).

T-cell receptors recognize the antigen bound to MHC molecules and activate T cells. Consequently, T_H cell secretes cytokines that contribute to the activation of B cells and T_c cells. Cytotoxic T lymphocytes recognize and kill altered self-cells. Lastly, the humoral response takes place and B cells interact with the antigens, differentiating into antibody-secreting plasma cells. Therefore, the antibody binds to the antigens and facilitates their clearance from the body (Horrocks, 2021; Khan Academy, 2017).

4. Transplants

The allograft transplant is the transfer between genetically different members of the same species. For allograft acceptance, the grafted epidermis must become revascularized, consequently, the blood supply is restored, and healing is promoted. The graft should be histocompatible to avoid an immunological reaction. MHC plays a key role to determine compatibility between the host and the donor. This is crucial for successful transplanting and avoiding rejection. The first step is to carry out tissue typing by using sequence-specific primers to identify similar donor and recipient alleles. In addition, to check for genetic similarities, it is important to check for antibodies to the potential donor in the host and this is done by the process of cross-matching (Horrocks, 2021).

Immunosuppressive therapy has been developed to ensure that a blood transfusion or organ transplant has a successful outcome. This can take several different forms. The most extreme is total lymphoid irradiation. Sites where lymphocytes aggregates such as the thymus, spleen, and lymph nodes are irradiated due to the sensibility of lymphocytes to X-rays. Then the transplant is made, and the sites are repopulated by lymphoid cells from the bone marrow, the immunological memory reset to 0 (Horrocks, 2021).

Another approach is to use general immunosuppression. This method uses azathioprine, or other compounds such as methotrexate, a mitotic inhibitor that inhibits both B and T cell proliferation, and consequently, the immune system response is reduced.

Cyclosporine A focuses only on blocking T-cell proliferation. General immunosuppression could have systemic side effects as it inhibits all dividing cells. Lastly, specific immunosuppression uses monoclonal antibodies against key immune proteins such as CD3 or T cell receptors. It can also be raised against key cytokines that play a role in the inflammatory response (Horrocks, 2021).

5. Conclusion

The immune system uses different molecules, such as MHC molecules, soluble factors, and opsins, to defend the body from any nonself material by recognising the antigen on the microorganism's cell surface. The phagocytes will then ingest, digest, and destroy it. The adaptive response coordinates T cells and B cells that form antigen-antibody complexes and kill altered self-cells. A successful transplant or blood transfusion can be helped by immunosuppressive therapy.

Cytolytic T-cells

1. Introduction

The immune system response is based on the interaction between the innate and adaptive system and their synergy. There are two different types of lymphocytes in the acquired immune system, B and T cells. The interaction between both types of cells leads to the activation and production of antibodies. MHC complexes are involved in the antigen recognition process by these lymphocytes. The two main groups of T lymphocytes are T helper T-cells (T_H) and T cytotoxic or cytolytic T-cells (T_c or CTL) (Horrocks, 2021; Raskov et al., 2020).

The main function of Cytolytic T- cells (CD8+ T cells) is to eradicate cells that show anomalous surface phenotype as a consequence of changes inside the cell. T lymphocytes are matured in the thymus and derived in the bone marrow from stem cells. CTL express CD8+ glycoproteins on their surface and are associated with MHC class I molecules. It could be damaging if they destroy antigen processing cells needed to create an immune response, limiting the production of antibodies. T-cells lyse cells with an antigen peptide on a surface MHC molecule. As a result of lysis, the cell membrane is broken down and the intercellular materials are released. The lymphocyte focuses on the H-2D and H-2K molecules on the cell, triggering the lytic signal (Doherty, 1980; Horrocks, 2021).

2. Low levels of CTL

Cytolytic T- cells are important to fight cancers and virus infection. They monitor cells of the host body, alert to destroy any threat. They kill virally infected cells, avoiding the

expansion of the viral pathogen. They can detect antigenic differences in altered cells, which helps to protect the host against malignant tumours. CTL recognise the antigen in the MHC class I, using the T-cell receptor and activating the pathways to kill target cells (Actor & Actor, 2012; Andersen et al., 2006).

Failure to protect the host from disease agents or malignancies is called immunodeficiency. Different diseases can develop in the lymphoid cell line depending on which agent is affected. In the case of lymphoid deficiencies, and especially T cells, the host will be more susceptible to viruses, fungi, and protozoa, as cytolytic T cells play a crucial role in combating these pathogens and infected host cells. They are also effectors in autoimmunity and immunoregulation functions. In addition, the various defects of T cells can affect the well-being of B cells and interfere in their communication. Consequently, cell surface receptors or the signal transduction molecules can be altered (Horrocks, 2021; Andersen et al., 2006).

Severe combined immunodeficiency affects T and B cells. It is characterized by low numbers of circulating lymphocytes, and it is usually fatal, as it is associated with failed T cell response (Horrocks, 2021). Malfunction of T cell regulation is involved in several diseases such as multiple sclerosis and insulin-dependent diabetes mellitus (Neumann et al., 2002). Vitiligo is a common autoimmune skin disease caused by the death of melanocytes by cytolytic T cells (Steitz et al., 2004).

NKT cells have been observed as a strong key factor for the immunosuppression of cytolytic T cells. The blocking of cytokines involved in CTL activation and induction such as IL-2 or IL-4 leads to the inhibition of cytolytic T cells activity (Ito & Seishima, 2010).

3. Risk of developing cancer

Immunotherapy works by using the host immune system to fight cancer. It uses different ways to boost the immune system to improve their chances of success in the killing process. T-cell transfer therapy takes T-cells from a patient and changed them in the laboratory to make T-cells better able to target the patient's cancer cells and kill them. Then, these modified cells are duplicated and given back to the patient to fight cancer (American cancer society, 2018).

Cytotoxic T cells are powerful effectors in cancer prevention and destruction of infected host cells. Suppressive immune receptors are engaged by immune checkpoint inhibitors to stimulate dysfunctional T lymphocytes, including CTL. This could cause a big impact on the cancer outcome (Stadtmauer et al., 2020; Raskov et al., 2020).

T lymphocytes navigate through almost all parts of the host to search for unwanted material. CTL interacts with the target cell by sustained mobility of the CD8+T cell on the infected cell. Mechanical forces intensify the formation of the pores and promote target cell death, as death-inducing granules are being secreted into the cell. The granule perforin damages the membrane of the target cell by creating holes. Then the granzymes get inside the cell and alter the proteins inside it, producing apoptosis of the target cell.

Moreover, cytolytic T- cells can express the Fas ligand so the binding between Fas and the target cell by Fas receptors stimulates death domains. In this way, endonucleases and caspases are activated and it leads to the disintegration of the target cell DNA (Raskov et al., 2020). Another method to fight infection is the activation of cytokines. The secretion of the cytokines IFN-gamma and TNF-alpha stimulates other cells from the immune system such as macrophages (Wissinger, no date).

If the patient has low levels of cytolytic T-cells, it will become much more vulnerable to cancer due to the low protection levels. If a tumour develops, no one will detect it, and it will continue to grow. Cold and hot tumours are the classification of cancer based on the number of cytolytic T-cells. Cold tumours present almost non-existent levels of CTL, failing to induce the contact between the antigen and the CD8 T cells.

4. Conclusion

Cytolytic T cells eliminate any cell or substance identified as harmful by the immune system. They play a big role in destroying virally infected cells and in fighting cancer. For this reason, low levels of CTL can leave the immune system vulnerable and without any protection from infected or altered cells.