The Unholy Trinity- Cancer cell plasticity, drug resistance and therapeutic opportunities

Namrata Chatterjee[1], Bhavana Pulipaka[2], Ayalur Raghu Subbalakshmi[3], Mohit Kumar Jolly[3], and Radhika Nair [4]

[1]National Institute of Technology Rourkela
[2]Ashoka University
[3]Indian Institute of Science
[4]Rajiv Gandhi Centre for Biotechnology

February 2, 2023

Abstract

Cancer cell plasticity is the ability of tumor cells to switch phenotypes and is one of the predominant requisites of cancer cells capable of undergoing metastasis. Cancer cell plasticity is also recognised as one of the major contributors to intra-tumoral heterogeneity, a critical factor underlying the progression of malignant tumors which is known to modify tumor response and induce resistance against various modes of therapy thus posing a barrier to efficient cancer management. Cancer cell plasticity is acquired by the subversion of cell signaling pathways like MAPK, PI3K, STAT3, Wnt, Hedgehog and Notch as well as cellular programs such as epithelial to mesenchymal transition (EMT) and phenotypic plasticity. This complex phenomenon has been studied in many cancer types like pancreatic cancer, colon cancer and breast cancer. Some cancers like breast cancer are known to exhibit a hierarchical organization with cancer stem cells (CSCs) at the pinnacle of the pyramid due to their ability to promote tumorigenesis, metastasis and therapy resistance. CSCs which can undergo phenotypic changes resulting in heterogenous cell populations with differing potential to develop programs necessary for the metastatic process lies at the core of the cancer cell plasticity hypothesis. Our expanding knowledge of this field can lead to the development of more therapeutic strategies that can be used in cancer and other solid tumors that exhibit similar mechanisms of plasticity. This review will explore the current understanding we have of breast cancer on the intrinsic molecular mechanisms of cancer cell plasticity and the resistance to various types of cancer therapy that arise as a result of plasticity. We conclude by exploring the potential novel therapies that specifically target the pathways leading to plasticity and can be leveraged to treat patients living with the disease. Keywords: Cancer cell plasticity, Epithelial to Mesenchymal Transition (EMT), therapy resistance, targeted therapy.

Introduction

The phenomenon of cancer cell plasticity – also known as phenotypic switching– is the phenomenon of specific genotypes producing varying different phenotypes in response to changing external cues. It has been widely studied as a critical process involved in development and evolution, but more recently, its role in the progression of cancer, metastasis, and resistance to therapy has been recognized. Cancer stem cells (CSCs) are linked with the discovery of phenotypic plasticity in cancer cells gave which hypothesizes the presence of a subset of cells in the cancer cell population that exhibit plasticity, self-renewal, differentiation and tumorigenicity. In addition to driving cancer progression and metastasis, CSCs have also been implicated in drug resistance which contributes to the aggressive growth of the tumor. Furthermore, the CSC paradigm has been proposed as a model to explain the phenomenon of intra-tumoral heterogeneity, which refers to the phenotypic, genetic and functional heterogeneity within the cell population of a single tumor as well as between primary and metastatic tumors that arise as a result of intrinsic genetic programs and extracellular triggers.

Cancer cell plasticity and the resultant phenomena of CSCs and intra-tumoral heterogeneity are particularly relevant in solid cancers like breast cancer, in which studies have shown that plasticity could arise as a result of epithelial to mesenchymal transition (EMT)[1] process induction that enables the carcinoma cells to metastasize to other locations within the body. Recent research has also found that factors such as genetic mutations, external stimuli or signaling from the tumor microenvironment as well as different environmental factors[2] can trigger the transformation of already differentiated cells into CSCs, indicating the presence of plasticity even at various stages of differentiation in breast cancer[3].

The molecular mechanisms that are related to the emergence of plasticity mainly attributes to intrinsic mechanisms involved in signaling pathways like Notch, MAPK, P13Kinase, Wnt, Hedgehog and STAT3 that work in tandem to control tumor cell dynamics and cell-to-cell communication. Cell-extrinsic factors like tumor microenvironment consisting of the immune system as well as tumor cell and the cancer-associated extracellular matrix (ECM) interaction, containing numerous receptors that have been strongly linked to providing a niche for cancer stem cells required for the development of plasticity.

In effective cancer treatment, therapy resistance poses a major problem, where tumor cells are initially responsive and develop resistance or have innate unresponsiveness to different anti-cancer drugs. Resistance in any type of cancer generally stems from several reasons - both genetic and epigenetic factors like cell plasticity, EMT, heterogeneity in cancer cells, mutations in different genes [4,5]. Alongside intrinsic factors and pathways facilitating drug resistance, the role of extrinsic factors like tumor microenvironment is equally crucial. Dynamic changes in the microenvironment of tumor contributes vastly to drug resistance. We acknowledge that the microenvironment is an area of interest, but will not be covering it in this review though there are some excellent review articles on this subject[6-8].

Common treatments that exist for different stages of cancer include surgery, hormonal therapy, radiation therapy, chemotherapy, targeted therapy and immunotherapy[9]. However, the major problem that stands in the way of an effective treatment regime is resistance to treatment. The need of the hour is to tackle the phenomenon of therapy resistance in cancer cells by targeting the intrinsic pathways as well as extrinsic factors that are seen to facilitate cell plasticity. Besides covering the mechanisms by which cell plasticity contributes to resistance, we will also focus on how we can target these pathways to negate therapeutic resistance with an overarching aim to obliterate cancer cells.

Cell intrinsic pathways involved in breast cancer plasticity

Cancer cell plasticity is induced by genetic factors and programs which lead to tumor progression, and metastasis to distant organs and confers therapeutic resistance to breast cancer cells. Major intrinsic cell signaling pathways like MAPK, PI3K/AKT/mTOR, Wnt, STAT3, Notch, Hedgehog and TGF-β have been found to be dysregulated in many cancer types and harbor the potential to induce cellular plasticity in cancer cells[10].

The MAPK pathway or Mitogen Activated Protein Kinase pathway[11] represents a cascade of protein kinases that are considered as the key signaling pathways that regulate cellular and molecular functions like proliferation, apoptosis, differentiation and stress response[12]. Emergence of MAPK pathway stems back 30 years ago[13]. Grouped into three families in mammals namely ERKs, p38/SAKs, JNKs [14], this pathway in the canonical form gets activated by an MAPK kinase kinase (MAPKKK). In the case of the ERK pathway of MAPK, Raf is the MAPKK which activates MAPKs MEK 1/2 which in turn mobilizes ERK 1/2. In JNK and p38 pathways of MAPK pathways, TAK1, MEKKs, MLKs and ASK1 act as MAPKKs leading to activation of MKK 4/7 and further downstream pathways involving Jun pathway and also MKK3/6 which activates p38[15]. Genetic mutation of the MAPK pathway contributes to 58% of tumors with different frequencies of mutations like 42.1% in the JNK pathway, 40.3% in p38, 33.7% in ERK 1/2 pathway and finally followed by only 6.1% in ERK5 pathway [16]. Aberrant activation of MAPK pathways and it's role in cancer progression and development may be due its role in the maintenance of CSCs in tumors. Loss of DUSP4 -dual specificity phosphate-4[10], a negative regulator of the MAP Kinase superfamily, leads to the development of phenotypes like CSCs in the basal type of breast cancer[17]. In another study, SIX1which

is a developmental homeoprotein activates the MAPK/ERK pathway leading to formation of CSC phenotype in luminal B breast cancer type[18]. Alongside aberrantly activated MAPK pathway also contributes in promoting EMT which in turn contributes to cancer cell plasticity. It is also associated with different disorders like cancer progression and fibrosis. ERK3 over-expression has also been observed to be associated with increase in cell migration and morphological changes of the breast cancer cells[19]. Mutations in BRAF gene lead to alterations in the RAS-RAF-MEK-ERK pathway[10] which has a major role in developing drug resistance. A pivotal transcriptional factor named FOXC1 is a factor that mediated cancer cell plasticity and metastasis via partial EMT which in turn leads to drug resistance in the same and is regulated by this signaling pathway in breast cancer cells[20].

The MAP-kinase pathway has a pivotal role in cellular proliferation in tumors like melanomas[21]. In the invasive and resistant melanoma cell lines, 229R and 238R, over-activation of MAPK was observed as a result of phenotypic switching. In oral squamous cell carcinoma (OSCC)[22], the MAPK pathway plays the role of an intermediary between the epithelial factor GRHL2 and the EMT-inducing signal TGFβ[23]. In the OSCC cell line SCC9, inhibition of MAPK signaling activated TGFβ signaling which in turn promoted the process of EMT. In the TNBC subtype, MAPK signaling is found to promote the enrichment of breast CSCs[24]. In SUM-159 and MDA-MB-231 cells, an abrogation of pluripotency factors SOX2, KLF4 and NANOG was seen upon the inhibition of p38-MAPK signaling pathway indicating its role in emergence of breast CSC phenotype. In breast ductal carcinoma, MAPK -ERK pathway was involved in the cytoskeletal reorganization and transition to a mesenchymal phenotype[25]. Rap1Gap, a GTPase-activating protein[26] suppression increased the levels of MAPK/ERK. MCF10A.DCIS cells show higher levels of Rap1Gap when compared to the invasive MCF10A.Ca1D. Rap1Gap silencing in MCF10A. DCIS cells resulted in elevated levels of ERK/MAPK which promoted their invasive capabilities accompanied by skeletal structure reorganization and gain of mesenchymal capabilities further outlining the role of the pathway in phenotypic switching. p44/p42 MAPK activation through Monocyte Chemoattractant Protein-1 (MCP-1)[27], which promotes EMT, resulted in an increase in cell invasion[28] in TNBC. In Py2T cells, the hyaluronan synthase (HAS2) gene was shown to modulate EMT and cell migration through Erk1/2 MAP-kinase signaling[29].

The second pathway that has a major role in cancer cell plasticity is PI3K/AKT/mTOR pathway. The PI3K/AKT/mTOR pathway[30]also known as the phosphatidylinositol 3 kinase/ protein kinase B pathway, is a highly conserved pathway affecting cellular processes such as proliferation, survival, growth, metabolism and immune response[31]. The signaling pathway came into prominence in 1977 with the discovery linking PI kinase activity with viral oncogene-encoded Tyr-kinase[32]. It also has an important role in various oncogenic regulations and thus targeting the PI3K pathway for therapeutic intervention has been a key research area in cancer biology.

In physiological conditions, activation happens by either receptor tyrosine kinases or GPCR (G protein-coupled receptors)[33]. Activation happens by phosphorylation of the phosphatidylinositol 4,5 biphosphate and converts it to phosphatidylinositol 3,4,5 trisphosphate. This phosphorylated PI3K phosphorylates the kinase domain of AKT at Threonine 308 residue which then binds to various downstream cytoplasmic as well as proteins facilitating the cell survival and growth. The next step to this canonical pathway involves the binding and phosphorylation by AKT to TSC2 which inhibits Rheb. Activated Rheb stimulates the mTOR complex 1 (mTORC1) which effects cell proliferation[34]. Occurrence of the pathway is observed in both the cytoplasm as well as the nucleus. The cytoplasmic counterpart of the pathway is considered the canonical route for regulating cellular functions. However, PI3K is also present in the nucleus[35]. Various factors target the pathway and are observed to aberrantly activate the pathway leading to progression of cancer in the patient for example the cancer-testis antigen A-kinase anchor protein 3 (AKAP3)[36]. In a study by Zhan et al through the use of tissue microarrays and immunohistochemistry, it was observed that AKAP3 was upregulated to a greater extent in cancerous cells of the breast and activates this pathway to facilitate cell migration, progression, migration in breast cancer cells[37]. This pathway in mediates cellular plasticity which results in the rise of the different cellular populations within the tumor which is documented in several studies like EMT induction that leads to repression of the tight junction proteins which mediates cell plasticity. In breast cancer, hormone receptor-positive tumors have been more associated with medi-

ating cellular plasticity and therapy resistance than breast cancer tumors which occur due to mutation in the PIK3CA gene[3]. Resistance of TNBC cells towards chemotherapeutic drugs has been observed due to mutation in the PI3K3CA pathway that encodes a subunit of PI3K pathway that causes inhibition of cell apoptosis, also facilitating cell proliferation [38]. Another study by Ghebeh et al. which focused on Fascin-mediated chemoresistance in breast cancer elucidated that PI3K/AKT pathway mediates the resistance[39]. The group used xenograft tumor models to evaluate effect of fascin in mediating chemoresistance in breast cancer cells. This experiment led to significant direct correlation of survival rate of patients who were treated with chemotherapy as a treatment modality and Fascin expression. The increase in chemoresistance of the cancer cells was also attributed to PI3K pathway in a parallel line to increased FAK phosphorylation, expression of XIAP and Livin which are inhibitors of apoptosis proteins, and also proapoptotic marker(caspase 9,3, PARP) suppression.

The PI3K pathway induces EMT directly or through its cooperation with other signaling pathways[40] . Activation of the pathway promotes occurrence of EMT during breast cancer progression[41] . Mitochondrial ribosomal protein L13 (MRPL13) was found to be a pro-EMT factor, as its depletion resulted in a decrease in mesenchymal markers like SNAI1 and VIM with an increase in the levels of ECAD. Further, it was observed that MRPL13 mediated EMT through PI3K/AKT/mTOR signaling pathway. Interestingly iit was observed that that the PI3K/AKT was involved in conferring the cells' resistance to Tamoxifen in addition to their role as promoters of EMT in T47D cells[42]. It was observed that when Connexin (Cx) 43, an EMT inhibitor was silenced alongside reduction in the levels of ECAD as well as an increase of NCAD levels. The addition of LY294002, a specific PI3K/Akt signaling inhibitor[43], suppressed EMT, corroborating role of the PI3K signaling in EMT progression. FAK/PI3K/Akt upregulation conferred the cells' resistance to Cisplatin in addition to promoting EMT[44] . Here it was observed that when MDA-M-231 cells and MCF-7 cells here treated with LY294002 it resulted in the attenuation of EMT with decreased levels of mesenchymal factors like SNAI1, SNAI2, VIM and NCAD and increase in the level of ECAD, an epithelial factor. Also, the treatment with LY294002 made cells sensitive to Cisplatin treatment. Analysis using T47D, MDA-MB-231 cell lines demonstrated that the EMT induction by Inorganic pyrophosphatase 1 (PPA1) mediated by the PI3K/AKT signaling pathway[45] . When the relationship between KRAS, PI3K/AKT and EMT was analyzed in T47D cells, it was seen that KRAS depletion resulted in the silencing of the signaling factors of PI3K/AKT, alongside EMT inhibition as indicated by the reduced NCAD levels and VIM with increased levels of ECAD[46].

STAT3 pathway is another major intrinsic cellular signaling pathway. The Signal Transducers and Activation of Transcription (STAT) 3 part of the STAT transcription factor family has seven highly conserved and structurally similar members. Since its discovery in 1994, STAT3, has been linked with cell growth and apoptosis, as well as in many disorders including different cancer types[47]. This signaling pathway generally gets activated by growth factors and cytokines leading to creating a docking site for cytoplasmic STAT3. After being activated by phosphorylation, homodimerization takes place from where they are translocated to the nucleus leading to binding to different DNA response elements and regulating their transcription[48]. This is one of the main intrinsic signaling pathways that in cancer gets aberrantly expressed and plays a major role in cancer cell inflammation. One such role of STAT3 pathway is observed in bladder cancer. In a study by Mirzaei et al, they found hyperactivation of STAT3 led to increased proliferation and metastatic ability of bladder cancer cells[49]. This is mainly due to the pathway mediating the inhibition of apoptosis and cell cycle arrest which leads to this aggressive behavior of the cancer cells. Another cancer type where STAT3 hyperactivation leads to enhanced cancer cell proliferation, tumor angiogenesis, invasion and metastasis is colorectal cancer[50]. STAT3 mediated key inflammatory factors and is excessively hyper-activated in colorectal cancer cells. Jung et al showed that STAT3 activated by Low-Density Lipoprotein Cholesterol mediates as well as enhances proliferation of cancer cells of the pancreas and prostate[51]. It was also observed in cell lines- PANC-1 and LNCaP where STAT3 pathways induced by LDL were deleted reducing the cell proliferation and migration. Similarly, STAT3 and its aberrant expression mediated breast cancer inflammation and proliferation.

The involvement of Wnt pathway in its canonical form is well-established, however more recent research has

uncovered the effect of the abnormal functioning of the pathway, through genetic and epigenetic defects, in the initiation, progression and development of drug-resistant characteristics in various cancers, through processes including the modulation of cancer stem cell behavior. The discovery of role of the Wnt pathway in cancer arose as a result of the finding that mammary tumors developing in mice after their infection with the murine mammary tumor virus were often due to the activation of the murine Int-1 gene, which was later named the Wnt-1. Later studies that revealed the relevance of the Wnt pathway in human cancers found that the cytosolic concentrations of β-catenin, a homolog of the Drosophila Armadillo protein with significant roles as the regulator of intracellular adhesion and as transcriptional activator controlled by the signaling pathway, was downregulated by a tumor-suppressor called adenomatous polyposis coli (APC) while the Wnt1 protein upregulated it, providing a basis for the Wnt/ β-catenin pathway-cancer connection. The aberrant functioning of the pathway can be observed amongst different cancers like colorectal, gastric, breast cancers,. Numerous genetically engineered mouse models have revealed that the induction of mammary tumorigenesis occurs as a consequence of the dysregulation of the Wnt/β-catenin pathway via the overexpression of its activators such as Wnt and the targeted disruption or silencing of its inhibitors such as APC.[52]

In most cancers, (most notably colorectal and gastrointestinal cancers) mutations that result in loss-of-function in the genes coding for the proteins involved in the Wnt pathway that is responsible for the degradation of beta-catenin such as APC and Axin - which are also tumor suppressor genes - have been recognized. As a result, the downregulation of the amount of free cytoplasmic beta-catenin is inhibited and the activation and transcription of the Wnt target genes ensue. In breast cancer, there has been growing evidence indicating the increased expression of pathway activators, suppression of tumor suppressor proteins and the enhanced transcription of Wnt target genes. For example, in transgenic mice with a mutation at the Int-1/Wnt-1 site, the formation of mammary adenocarcinomas with metastasis to the lymph nodes was discovered. Additionally, the mammary tumorigenesis in the same transgenic mice also showed cooperation with various oncogenes coupled with the suppression of several tumor suppressor genes such as the p53 gene. Similarly, in a different mice model carrying the Apc Min/+ allele, which coded for a truncated version of the APC protein, it was discovered that the females carrying the mutation were more likely to develop spontaneous mammary tumors and even showed enhanced sensitivity to mammary carcinogenic compounds. Moreover, various studies have also brought to light that the over-expressed target genes of Wnt/beta-catenin like the c-myc, MMP-3, MMP-7 genes in mammary epithelial cells leads to tumorigenesis. An additional aspect of breast cancer progression where a critical role of Wnt pathway is observed is in the maintenance of plasticity BSCS. Recent research has highlighted that the PCNA-associated factor (PAF), which is seen to control cancer cell stemness, interacts with the Wnt signaling by hyperactivating it via serving as a co-factor of the beta-catenin transcription complex within the nucleus and thereby increasing Wnt target gene expression. This connection was discovered when it was found that PAF expression in mammary epithelial cells upregulated beta-catenin as well as enhanced its translocation into the nucleus[53].

Under physiological conditions, the hedgehog (Hh) pathway plays a vital role in embryonic development by patterning the developing neural tube, axial skeleton, limbs, skin, lungs, hair and teeth. However, the contribution of the aberrant form of the pathway to tumorigenesis is being made clear by accumulating evidence in recent years. However, the first clue connecting the hedgehog signaling pathway to cancer came as a result of the discovery that a mutation in the tumor suppressor gene that encodes for a receptor molecule found within the pathway called patched (Ptc) was an underlying factor in the development of both familial and sporadic forms of basal cell carcinoma (BCC).

The Notch pathway is a tightly controlled ssignaling pathway responsible for various aspects of development of metazon as well as renewal of tissue. As a conserved signaling pathway, the Notch pathway plays a critical role in short-range communication between cells through the establishing physical contact between the target cells. The pathway is responsible for enabling and suppressing, depending on context and molecular cues, and cellular functioning like cell differentiation, proliferation of cells, cell death and controlling cell fates throughout the development of the organism as well as in self-renewing adult tissue. Given the vital role it plays, the mis-regulated loss-of or gain-of function of the Notch pathway can give rise to various human diseases, including cancer. Given the fundamental role played by Notch in determination of cell fate and the

maintenance of adult stem cells and progenitor cells, its dysregulation has been linked to the maintenance of cancer stem cells (CSC) which, as growing evidence suggests, could be the initiator of tumorigenesis, according to the cancer stem cell model. In breast cancer specifically, studies have shown that Notch acts as an oncogene. The overexpression of the Notch proteins 1, 3 or 4 has been shown to transform normal breast epithelial cells to cancer cells, leading to the overactivation of the signaling pathway.

Drug Resistance Mediated by Cellular Plasticity

While chemotherapy is the mainstay in our battle against cancer, either intrinsic or extrinsic reasons leads to the cancer cells acquiring the ability of evading the effect of drugs[54]. One of the main factors for drug resistance or therapy resistance is cellular plasticity. Different pathways as discussed above have been shown to be involved in the phenomenon of cellular plasticity leading resistance towards drugs. An example is up regulation of the p44/MAPK pathway by cytokine TGF-β1, released from the chemo resistant cells and which induces resistance in breast cancer cells. A recent study has linked CD24, a CSC maker to phenotypic switching and thus induction of cellular plasticity to the MAPK pathway. The cellular localization of CD24 is mediated by p38 MAPK pathway activation with phosphorylation leading to Bcl over-expression resulting in the localization of a high-density of CD24 molecules in the cell membrane[55] and treatment resistance. Non genomic factors like Estrogen have been shown to affect mediating cellular plasticity in the tumor cells that have a propensity towards undergoing EMT. The resistance is mediated by c-Src activation which in turn mediates activation of cell signaling pathways like MAPK[56]-. Targeting these factors can yield a therapeutic intervention point to check the treatment resistance mediated by cellular plasticity.

Radio-therapy is the use of radiation to curb growth and proliferation of the cancer cells. Effectiveness of radiotherapy is curtailed by adaptive radioresistance or the acquisition of resistance by the cells over a time period. In radioresistant breast cancer cell line the major reason for driving radioresistance was increase in cancer stem cells (CSCs) alongside elevated EpCAM expression. It was observed that elevated EpCAM expression conferred resistance towards radiotherapy in breast cancer cell line ZR-75-1 by enhancing Akt expression which in turn increases the stemness of the cells. Thus, this pathway act as a driving factor for EpCAM mediated cellular plasticity in the breast cancer cell line [57].

A commonly upregulated factor CDLYL2 in breast cancer is observed to mediate the cell plasticity phenomenon by regulating miRNA-124 which in turn impacts both STAT3 and NF-ϰβ pathways[58]. The over-expression of the CDYL2 lead to changes at morphological and molecular level in the breast cancer cells. Another study by Arnold et al. found radiation to be responsible for conferring radiation-mediated cellular plasticity in breast cancer stem-like cells alongside induction of radioresistance in those cells[59]. The findings of these researchers confirmed that conferring resistance and also changes in cellular plasticity in the TNBC cell line were mediated by STAT3 via an inflammatory response. The inflammatory response that triggers the cellular pathway is mediated by IL6. This interleukin binds to its own receptors which lead to the activation of Janus family kinase which in phosphorylates and triggers STAT3 pathway. Resistance towards the drug Lapatinib in locally advanced HER2+ breast cancer is mainly mediated by IL-8 that is secreted from tumor macrophages via activation of Src/STAT3/ERK1/2 pathway which in turn mediated EGFR signalling[60]. This pathway is observed to confer radio-resistance to breast cancer cell lines can be attributed to the activation of signal transduction by activation of the STAT3 pathway by phosphorylation of Tyr705 residue of the signal transducer[61]. Thus, targeting STAT3 will probably yield inhibition of the phenomenon of drug resistance in breast cancer.

Not only does a hyperactivated Wnt pathway and the resultant dysregulation of the proteolytic degradation of beta-catenin lead to tumorigenesis, but it is also strongly linked to the development of drug resistance

seen in many invasive cancers. Conventional chemotherapy affects cancer cells by exposing them to cytotoxic agents that inhibit cell proliferation and induce cell death in rapidly-proliferating cells while radiotherapy involves the utilization of ionizing radiation to tumor cells, damaging their DNA and causing cell death as a consequence. CSCs are generally known to be more resistant to chemo and radiation therapies as compared to the non-stem cell populations in the tumor, allowing them to induce a cancer relapse even after such treatments. Given that the Wnt signaling pathway plays an important role in maintaining cancer stem cells, it could be seen as a major mechanism underlying the development of therapeutic resistance in many cancer types. For example, in a study using carboplatin-resistant in-vivo patient-derived xenografts as well as isogenic triple-negative breast cancer (TNBC) cell-line models, it was found that there was an increased activation of the Wnt/beta-catenin pathway which was correlated with the expression of stem cell markers in both the models studied. Moreover, this study also proved that the inhibition of the Wnt signaling pathway in these models resulted in their resensitization to carboplatin from their resistant states – thus highlighting a potential mechanism for overcoming chemotherapeutic resistance, especially in breast cancer[62]. Similarly, another study found that a protein called ST8SIA1 and its corresponding mRNA was upregulated in chemotherapy-resistant TNBC patients and that the inhibition of the ST8SIA1 mRNA led to the increase in the efficacy of chemotherapy through the suppression of the Wnt/beta-catenin pathway – thus linking the signaling pathway to the development of chemotherapeutic resistance, particularly in breast cancer[63]. In addition, it has also been found that chemotherapy and radiation can upregulate the Wnt/beta-catenin pathway even in non-cancer stem cell populations - thus upregulating the genes that protect the cells from cell cycle arrests and apoptosis as well as enhancing DNA damage repair which reverses the effects of chemotherapeutic drugs[64]. Immune evasion by ER+ breast cancer cells has also been shown to be mediated by the Wnt signaling pathway. For example, a recent study used microarray-based gene expression studies to identify the overexpression of a microRNA – miR-18a – involved in the suppression of immune-related pathways in ER+ breast cancer cell lines. The function of this particular miRNA was also found to be linked to the Wnt pathway as the inhibition of the signaling pathway in miR-18a over-expressed cells resulted in the restoration of antigen presentation properties[65]. Additionally, another study showed that the activation of the Wnt/bata-catenin pathway led to the absence of a T-cell gene expression signature which thereby caused a lack of T-cell infiltration in human metastatic melanoma samples. Furthermore, the Wnt pathway is also shown to have a role in the suppression of tumor cell-intrinsic cytokines which inhibits the recruitment of CD103+ dendritic cells and the activation of the CD8+ T-cells[64].

The Hh connection to cancer was first unearthed when a mutation in the Ptc gene was linked to BCC, also known as Gorlin's syndrome. Following this discovery, the Ptc mutation has been found in other Gorlin's syndrome-related tumors such as meningioma, medulloblastoma and even other cancers like breast cancer and transitional cell carcinoma. From a functional perspective, the mutation of the Ptc gene leads to the inactivation of the normal Ptc protein and causes the hyperactivation of the Hh pathway due to the enhanced function of Smo, thereby leading to the overexpression of the Hh target genes. Additional mutations of the Smo gene as well as the overexpression of the hh gene have also been identified in vivo and in vitro models.

One of the initial theories to explain the phenomenon of drug resistance in breast cancer cites breast cancer stem cells (BCSCs), which are thought to be inherently resistant to cytotoxic and targeted therapies. Recent research has been able to find new evidence that links Notch1 and Notch4 in the development of drug-resistant behavior exhibited by BCSCs. A study using a mammary tumor mouse model also showed that cells showed a temporal increase in expression of the Notch ligand, DII1, as the tumor progressed from early to late stages. Moreover, these DII1+ cells also exhibit chemotherapeutic resistant gene signatures and the consequent blocking of the ligand sensitized the cells to chemotherapy as well as stopping tumor growth and metastasis[66]. Moreover, the small interfering RNA (siRNA)- mediated knockdown of Notch1 in ALDH+ cells, resulted in an inhibition of their growth and increased apoptosis and tumor growth in mice models[67].

The molecular mechanisms that contribute to the phenomenon of resistance remain to be elucidated completely. Yet research on the same has paved the way for targeting various crucial factors that have been seen to play a role in conferring cellular plasticity and therapy resistance in breast cancer cells. These factors can thus act as potential biomarkers for targeted therapy and new treatment modalities can be developed on the

same.

(Figure 1): Cell intrinsic pathways involved in cancer cell plasticity.

Potential treatments targeting cell-intrinsic pathways mediating cancer cell plasticity

One of the most targeted proteins in the MAPK pathway is the MEK protein. MEK targeting drugs have been observed to lead to improvement in the prognosis of breast cancer patients. One such targeted therapy involved a combinational therapy of RAF/MEK inhibitor CH5126766 alongside Eribulin in TNBC and it was observed that this combinational mechanism was successful in inhibiting apoptosis and cell migration, thus contributing to tumor growth[68]. In another study, dual inhibition of MEK1/2 and MEK 5 by using inhibitors Cobimetinib and Trametinib resulted in the suppression of EMT in triple negative breast cancer[69]. Mohan et al have also shown that Brucein D (BD) which is an inhibitor targeting the p38 pathway lead to apoptosis and decrease viability of the breast cancer cells induced by the MAPK pathway[70]. Alongside MEK inhibitors, the use of RNA binding proteins has also been elucidated. In one study, RNA binding protein QKI was observed to suppress the breast cancer cell progression by directly binding to RASA 1 and activating the MAPK signaling pathway[71]

PI3K/AKT/mTOR pathway accounts as an altered pathway in breast cancer cell plasticity. Inhibition of the pathway using different small molecule inhibitors targeting different components of the pathway has emerged as one of the prime research interests among researchers. Various different small inhibitors that target the PI3K pathway are being looked into as potential treatment alternatives. *In vitro*studies on inhibitors like Buparlisib, Pictilisib, Alpelisib, Taselisib have led to the pro-apoptotic and anti-proliferative activity on breast cancer cell lines[72]. Natural compounds have also started to gain popularity for potential targeting of the pathway. One such bibenzyl compound is Gigantol extracted from orchids belonging to the *Dendrobium* genus. It has been observed to downregulate pathway in cancer cells thus in turn inhibiting proliferation and induce apoptosis. The study involved in silico determination of the potential pathway by which Gigantol acts followed by in vitro assays to elucidate effect of the same on breast cancer cell line[73]. Another natural compound that has emerged as a potential candidate for therapy in TNBC is a novel Anilide Shikonin ester- M9. Ma et al. elucidated effect of M9 on breast cancer cell line MDA-MB-231 and observed that M9 has the ability to induce apoptosis and arrest cell cycle by affecting both PI3K/Akt/m-TOR pathway and Wnt/β-catenin pathway. It inhibits Akt thus downregulating the PI3K pathway[74]. Inhibitors of Akt are the foremost therapeutic intervention points that is observed to promote anti cancer effect in breast cancer cells. Such a study was done by Zhu et al., where they observed that the effect of AZD5363, an Akt inhibitor was successful in reducing stemness of the breast cancer cell line MCF-7. It was used in a combinatorial approach alongside 3,3'-diindoylmethane and it was also observed that the anti-cancer effect of the drug and induced DIM induced apoptosis in the cells[75]. Luminal A breast cancer cells are again a potential biomarker for modifying the phenotypic plasticity amongst the cells leading to aggressive and inducting resistance towards therapy. Dietary phytochemicals both alone and in an combinatorial approach has been used as a therapeutic modality targeting the luminal cells via modulating the key signalling pathways including PI3k-AKT-mTOR pathway, MAPK pathway, Notch pathway, Hedgehog pathway and others[76].

Signal Transducer 3 and its upregulation in mediating cell plasticity and therapy resistance in breast cancer which makes it one of the potent targets for small molecule inhibitors and drugs. One such anti-cancer inhibitor, a natural phytoalexin, Resveratrol regulates the cascade of STAT signalling and has the potential to mitigate breast cancer progression[77]. Another study on anti-cancer drug resistance, trastuzumab which

targets HER2+ cancer develops resistance in some patients. In order to circumvent this problem scientists have targeted proto-oncogene MUC1 which is one of the downstream targets of STAT3[78]. 6Br-6a is a STAT3 inhibitor that has been evaluated as a therapeutic advancement as it inhibits STAT3 activation and induces inhibition towards cell proliferation, induce apoptosis and arrest of the cell cycle in breast cancer cells. Thus it can act as a promising target for breast cancer [79].

The use of molecules that inhibit the various constituents of the Wnt signaling cascade is currently being studied as a potential therapeutic strategy for Wnt-mediated cancers and as a mechanism to inhibit the therapy resistance conferred by this oncogenic signaling pathway. For example, the introduction of a truncated version of the APC protein was shown to have moderate success in mediating the degradation of beta-catenin in colorectal cancers, where the APC gene has a loss-of-function mutation. Another potential mechanism to elevate the beta-catenin degradation involves the increase in Axin production through the active inhibition of tankyrases, which as proteins that drive the degradation of Axin. Tankyrase inhibitors such as XAV939 have shown strong suppression of the Wnt/beta-catenin pathway, resulting in an anti-cancer effect in Wnt-mediated cancers[64].

Given the prevalent role of the Hh signaling pathway in various types of cancers, its inhibition can be a strong contender as a potential therapeutic strategy. One major mechanism through which Hh pathway targeting therapies can be created is through the development of inhibitor molecules that target the pathway and silence it. For example, Smo inhibitors that target the Smo receptor have proved to be fairly successful. Two different Smo inhibitors, vismodegib and sonidegib, have received approval from the FDA for the treatment of BCC after it was found that these molecules were able to successfully bind to Smo and inhibit the downstream activation of the Hh target genes. Furthermore, more novel Smo inhibitors such as Saridegib and Taladegib are now under development, demonstrating the scope in the area of Smo inhibitor-based treatment mechanisms for several cancer types[80]. Moreover, cyclin-dependent kinase (CDK) inhibitors such as dinaciclib are also currently being tested in clinical trials against various types of cancer, including breast cancer. A recent study was able to show that the CDK inhibitors were able to reduce stemness and other malignant properties in TNBC cells by targeting the Hh signaling pathway[81].

Targeted therapies involving the inhibition of the hyperactive Notch pathway in breast cancer are chiefly based on the use of gamma-secretase inhibitors, transcriptional inhibitors as well as the use of monoclonal antibodies to target the various ligand and receptor components of the Notch pathway. For example, in vitro studies with ER+ breast cancer cells showed that the use of the gamma-secretase inhibitor Z-Leu-Leu-Nle-CHO[82] led to the strong inhibition of its growth. Similarly, the use of the monoclonal antibody OMP-59R5[83], designed to specifically target the Notch2 and 3 receptors has shown increased disease efficacy in clinical trials of triple-negative breast cancer. Furthermore, a study using a TNBC murine model showed that the inhibition of a deubiquitinase USP9x using a small molecule inhibitor called G9, reduces Notch activity[84] and consequently remodels the tumor landscape and decreases tumor growth.

(Table 1)

Conclusion and Future Perspectives

Interest in understanding cellular plasticity in different forms of cancer as well as its crucial role in therapy resistance has led to uncovering the mechanism and targeting different major cell-intrinsic as well as extrinsic drivers of the process. The main focus of the review is the cell intrinsic pathways that modulate the phenomenon of cell plasticity. Cell intrinsic mechanisms include major signaling pathways which are mostly dysregulated in cancer and tend to inculcate plasticity in the breast cancer cells[10,85]. These dysregulated pathways also are major driving factors for resistance towards treatment and targeting the same has been the major focus of the researchers for a long time. This had led to various targeted therapy and the development of several inhibitors of those dysregulated biomarkers.

(Figure 2): Targeting molecular pathways contributing to cancer cell plasticity

Another important player that has a major role in cellular plasticity induction and therapy resistance is the

tumor microenvironment (TME)[86]. The complex networking of different cells and immune system players along with the cancer cells forms an intricate connection and provides an avenue for inducting cellular plasticity as well as therapy resistance due to the same. Targeting the microenvironment will also lead to potential therapeutic avenues for curbing this deadly phenomenon of plasticity and therapy resistance. The exact mechanisms both intrinsic and extrinsic mechanisms leading to cellular plasticity and therapy resistance in cancer are yet to be untangled. This will profit the researchers who are continuously looking for designing therapeutic approaches that aim at perturbing the interplay of cellular factors resulting in plasticity and restricting it alongside curbing tumor heterogeneity[87] caused by the same which accounts for therapy resistance.

References

1 Giuli, M. V., Mancusi, A., Giuliani, E., Screpanti, I. & Checquolo, S. Notch signaling in female cancers: a multifaceted node to overcome drug resistance. *Cancer Drug Resistance* **4** , 805-836, doi:10.20517/cdr.2021.53 (2021).

2 Fusco, G. & Minelli, A. Phenotypic plasticity in development and evolution: facts and concepts. Introduction. *Philos Trans R Soc Lond B Biol Sci* **365** , 547-556, doi:10.1098/rstb.2009.0267 (2010).

3 Smigiel, J. M. *et al.* Cellular plasticity and metastasis in breast cancer: a pre- and post-malignant problem. *J Cancer Metastasis Treat* **5** , doi:10.20517/2394-4722.2019.26 (2019).

4 Mansoori, B., Mohammadi, A., Davudian, S., Shirjang, S. & Baradaran, B. The Different Mechanisms of Cancer Drug Resistance: A Brief Review.*Adv Pharm Bull* **7** , 339-348, doi:10.15171/apb.2017.041 (2017).

5 Shen, S. & Clairambault, J. Cell plasticity in cancer cell populations. *F1000Res* **9** , doi:10.12688/f1000research.24803.1 (2020).

6 Faurobert, E., Bouin, A.-P. & Albiges-Rizo, C. Microenvironment, tumor cell plasticity, and cancer. *Current Opinion in Oncology***27** , 64-70, doi:10.1097/cco.0000000000000154 (2015).

7 Zhang, R., Tu, J. & Liu, S. Novel molecular regulators of breast cancer stem cell plasticity and heterogeneity. *Seminars in Cancer Biology* **82** , 11-25, doi:https://doi.org/10.1016/j.semcancer.2021.03.008 (2022).

8 Wang, X. & Thiery, J. P. Harnessing Carcinoma Cell Plasticity Mediated by TGF-β Signaling. *Cancers* **13** , 3397 (2021).

9 Moo, T. A., Sanford, R., Dang, C. & Morrow, M. Overview of Breast Cancer Therapy. *PET Clin* **13** , 339-354, doi:10.1016/j.cpet.2018.02.006 (2018).

10 Kong, D., Hughes, C. J. & Ford, H. L. Cellular Plasticity in Breast Cancer Progression and Therapy. *Front Mol Biosci* **7** , 72, doi:10.3389/fmolb.2020.00072 (2020).

11 Mohapatra, T. & Dixit, M. IQ Motif Containing GTPase Activating Proteins (IQGAPs), A-Kinase Anchoring Proteins (AKAPs) and Kinase Suppressor of Ras Proteins (KSRs) in Scaffolding Oncogenic Pathways and Their Therapeutic Potential. *ACS Omega* **7** , 45837-45848, doi:10.1021/acsomega.2c05505 (2022).

12 Guo, Y. J. *et al.* ERK/MAPK signalling pathway and tumorigenesis (Review). *Exp Ther Med* **19** , 1997-2007, doi:10.3892/etm.2020.8454 (2020).

13 Ahn, N. G. The MAP kinase cascade. Discovery of a new signal transduction pathway. *Molecular and Cellular Biochemistry***127** , 201-209, doi:10.1007/BF01076771 (1993).

14 Morrison, D. K. MAP kinase pathways. *Cold Spring Harb Perspect Biol* **4** , doi:10.1101/cshperspect.a011254 (2012).

15 Turner, N. & Blythe. Cardiac Fibroblast p38 MAPK: A Critical Regulator of Myocardial Remodeling. *Journal of Cardiovascular Development and Disease* **6** , 27, doi:10.3390/jcdd6030027 (2019).

16 Sinkala, M., Nkhoma, P., Mulder, N. & Martin, D. P. Integrated molecular characterisation of the MAPK pathways in human cancers reveals pharmacologically vulnerable mutations and gene dependencies.*Communications Biology* **4** , 9, doi:10.1038/s42003-020-01552-6 (2021).

17 Balko, J. M. *et al.* Activation of MAPK Pathways due to DUSP4 Loss Promotes Cancer Stem Cell-like Phenotypes in Basal-like Breast CancerDUSP4 Loss Promotes CSC Phenotypes in Basal-like Breast Cancer.*Cancer research* **73** , 6346-6358 (2013).

18 Iwanaga, R. *et al.* Expression of Six1 in luminal breast cancers predicts poor prognosis and promotes increases in tumor initiating cells by activation of extracellular signal-regulated kinase and transforming growth factor-beta signaling pathways. *Breast Cancer Research* **14** , 1-14 (2012).

19 Al-Mahdi, R. *et al.* A novel role for atypical MAPK kinase ERK3 in regulating breast cancer cell morphology and migration. *Cell Adhesion & Migration* **9** , 483-494, doi:10.1080/19336918.2015.1112485 (2015).

20 Ray, T., Ryusaki, T. & Ray, P. S. Therapeutically Targeting Cancers That Overexpress FOXC1: A Transcriptional Driver of Cell Plasticity, Partial EMT, and Cancer Metastasis. *Front Oncol* **11** , 721959, doi:10.3389/fonc.2021.721959 (2021).

21 Sala, M. *et al.* Discoidin Domain Receptor 2 orchestrates melanoma resistance combining phenotype switching and proliferation.*Oncogene* **41** , 2571-2586, doi:10.1038/s41388-022-02266-1 (2022).

22 Valipour, M. Recent advances of antitumor shikonin/alkannin derivatives: A comprehensive overview focusing on structural classification, synthetic approaches, and mechanisms of action.*European Journal of Medicinal Chemistry* **235** , 114314, doi:https://doi.org/10.1016/j.ejmech.2022.114314 (2022).

23 Chen, W. *et al.* Grainyhead-like 2 (GRHL2) knockout abolishes oral cancer development through reciprocal regulation of the MAP kinase and TGF-β signaling pathways. *Oncogenesis* **7** , 38, doi:10.1038/s41389-018-0047-5 (2018).

24 Lu, H. *et al.* Reciprocal Regulation of DUSP9 and DUSP16 Expression by HIF1 Controls ERK and p38 MAP Kinase Activity and Mediates Chemotherapy-Induced Breast Cancer Stem Cell Enrichment. *Cancer Research* **78** , 4191-4202, doi:10.1158/0008-5472.Can-18-0270 (2018).

25 Shah, S. *et al.* Downregulation of Rap1Gap: A Switch from DCIS to Invasive Breast Carcinoma via ERK/MAPK Activation. *Neoplasia (New York, N.Y.)* **20** , 951-963, doi:10.1016/j.neo.2018.07.002 (2018).

26 Wu, M. & Zhao, H. Analysis of key genes and pathways in breast ductal carcinoma in situ.*Oncol Lett* **20** , 217, doi:10.3892/ol.2020.12080 (2020).

27 17th International Congress of Immunology, 19–23 October 2019, Beijing, China. *European Journal of Immunology* **49** , 1-2223, doi:https://doi.org/10.1002/eji.201970400 (2019).

28 Dutta, P., Sarkissyan, M., Paico, K., Wu, Y. & Vadgama, J. V. MCP-1 is overexpressed in triple-negative breast cancers and drives cancer invasiveness and metastasis. *Breast Cancer Research and Treatment***170** , 477-486, doi:10.1007/s10549-018-4760-8 (2018).

29 Kolliopoulos, C., Lin, C. Y., Heldin, C. H., Moustakas, A. & Heldin, P. Has2 natural antisense RNA and Hmga2 promote Has2 expression during TGFβ-induced EMT in breast cancer. *Matrix Biol* **80** , 29-45, doi:10.1016/j.matbio.2018.09.002 (2019).

30 BeLow, M. & Osipo, C. Notch Signaling in Breast Cancer: A Role in Drug Resistance. *Cells* **9** , 2204 (2020).

31 Shi, X. *et al.* Research progress on the PI3K/AKT signaling pathway in gynecological cancer (Review). *Mol Med Rep***19** , 4529-4535, doi:10.3892/mmr.2019.10121 (2019).

32 Vanhaesebroeck, B., Stephens, L. & Hawkins, P. PI3K signalling: the path to discovery and understanding. *Nature Reviews Molecular Cell Biology* **13** , 195-203, doi:10.1038/nrm3290 (2012).

33 Beyens, M., Vandamme, T., Peeters, M., Van Camp, G. & Op de Beeck, K. Resistance to targeted treatment of gastroenteropancreatic neuroendocrine tumors. *Endocrine-Related Cancer* **26** , R109-R130, doi:10.1530/erc-18-0420 (2019).

34 Tran, P., Nguyen, C. & Klempner, S. Targeting the Phosphatidylinositol-3-kinase Pathway in Gastric Cancer: Can Omics Improve Outcomes? *International Neurourology Journal* **20** , S131-140, doi:10.5213/inj.1632740.370 (2016).

35 Gupta, S., Kumar, M., Chaudhuri, S. & Kumar, A. The non-canonical nuclear functions of key players of the PI3K-AKT-MTOR pathway.*Journal of Cellular Physiology* **237** , 3181-3204, doi:https://doi.org/10.1002/jcp.30782 (2022).

36 Nicolini, A., Ferrari, P. & Duffy, M. Prognostic and Predictive Biomarkers in Breast Cancer: Past, Present and Future. *Seminars in Cancer Biology* **52** , doi:10.1016/j.semcancer.2017.08.010 (2017).

37 Zhan, C.-H. *et al.* The cancer-testis antigen a-kinase anchor protein 3 facilitates breast cancer progression via activation of the PTEN/PI3K/AKT/mTOR signaling. *Bioengineered* **13** , 8478-8489, doi:10.1080/21655979.2022.2051687 (2022).

38 Hu, H. *et al.* PIK3CA mutation confers resistance to chemotherapy in triple-negative breast cancer by inhibiting apoptosis and activating the PI3K/AKT/mTOR signaling pathway. *Ann Transl Med* **9** , 410, doi:10.21037/atm-21-698 (2021).

39 Ghebeh, H. *et al.* Fascin is involved in the chemotherapeutic resistance of breast cancer cells predominantly via the PI3K/Akt pathway. *British Journal of Cancer* **111** , 1552-1561, doi:10.1038/bjc.2014.453 (2014).

40 Xu, W., Yang, Z. & Lu, N. A new role for the PI3K/Akt signaling pathway in the epithelial-mesenchymal transition. *Cell Adh Migr***9** , 317-324, doi:10.1080/19336918.2015.1016686 (2015).

41 Cai, M., Li, H., Chen, R. & Zhou, X. MRPL13 Promotes Tumor Cell Proliferation, Migration and EMT Process in Breast Cancer Through the PI3K-AKT-mTOR Pathway. *Cancer Manag Res* **13** , 2009-2024, doi:10.2147/cmar.S296038 (2021).

42 Wu, D. P. *et al.* Cx43 deficiency confers EMT-mediated tamoxifen resistance to breast cancer via c-Src/PI3K/Akt pathway.*Int J Biol Sci* **17** , 2380-2398, doi:10.7150/ijbs.55453 (2021).

43 Wu, D.-P. *et al.* Cx43 deficiency confers EMT-mediated tamoxifen resistance to breast cancer via c-Src/PI3K/Akt pathway.*International Journal of Biological Sciences* **17** , 2380-2398, doi:10.7150/ijbs.55453 (2021).

44 Luo, J. *et al.* 14, 15-EET induces breast cancer cell EMT and cisplatin resistance by up-regulating integrin αvβ3 and activating FAK/PI3K/AKT signaling. *Journal of Experimental & Clinical Cancer Research* **37** , 23, doi:10.1186/s13046-018-0694-6 (2018).

45 Guo, C. *et al.* PPA1 Promotes Breast Cancer Proliferation and Metastasis Through PI3K/AKT/GSK3β Signaling Pathway. *Front Cell Dev Biol* **9** , 730558, doi:10.3389/fcell.2021.730558 (2021).

46 Zhang, Y., Liu, J. L. & Wang, J. KRAS gene silencing inhibits the activation of PI3K-Akt-mTOR signaling pathway to regulate breast cancer cell epithelial-mesenchymal transition, proliferation and apoptosis.*Eur Rev Med Pharmacol Sci* **24** , 3085-3096, doi:10.26355/eurrev_202003_20673 (2020).

47 Ma, J.-h., Qin, L. & Li, X. Role of STAT3 signaling pathway in breast cancer. *Cell Communication and Signaling* **18** , 33, doi:10.1186/s12964-020-0527-z (2020).

48 Johnston, P. A. & Grandis, J. R. STAT3 signaling: anticancer strategies and challenges. *Mol Interv* **11** , 18-26, doi:10.1124/mi.11.1.4 (2011).

49 Mirzaei, S. *et al.* Pre-clinical investigation of STAT3 pathway in bladder cancer: Paving the way for clinical translation.*Biomedicine & Pharmacotherapy* **133** , 111077, doi:https://doi.org/10.1016/j.biopha.2020.111077 (2021).

50 Gargalionis, A. N., Papavassiliou, K. A. & Papavassiliou, A. G. Targeting STAT3 Signaling Pathway in Colorectal Cancer.*Biomedicines* **9** , 1016 (2021).

51 Jung, Y. Y. *et al.* LDL cholesterol promotes the proliferation of prostate and pancreatic cancer cells by activating the STAT3 pathway.*Journal of Cellular Physiology* **236** , 5253-5264, doi:https://doi.org/10.1002/jcp.30229 (2021).

52 Polakis, P. Wnt signaling in cancer. *Cold Spring Harb Perspect Biol* **4** , doi:10.1101/cshperspect.a008052 (2012).

53 Prosperi, J. R. & Goss, K. H. A Wnt-ow of opportunity: targeting the Wnt/beta-catenin pathway in breast cancer. *Curr Drug Targets***11** , 1074-1088, doi:10.2174/138945010792006780 (2010).

54 Alfarouk, K. O. *et al.* Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. *Cancer Cell Int***15** , 71, doi:10.1186/s12935-015-0221-1 (2015).

55 Huth, H. W., Castro-Gomes, T., de Goes, A. M. & Ropert, C. Translocation of intracellular CD24 constitutes a triggering event for drug resistance in breast cancer. *Scientific Reports* **11** , 17077, doi:10.1038/s41598-021-96449-7 (2021).

56 Jiménez-Salazar, J. E., Damian-Ferrara, R., Arteaga, M., Batina, N. & Damián-Matsumura, P. Non-Genomic Actions of Estrogens on the DNA Repair Pathways Are Associated With Chemotherapy Resistance in Breast Cancer. *Front Oncol* **11** , 631007, doi:10.3389/fonc.2021.631007 (2021).

57 Mal, A. *et al.* EpCAM-Mediated Cellular Plasticity Promotes Radiation Resistance and Metastasis in Breast Cancer. *Front Cell Dev Biol* **8** , 597673, doi:10.3389/fcell.2020.597673 (2020).

58 Siouda, M. *et al.* CDYL2 Epigenetically Regulates MIR124 to Control NF-ϰB/STAT3-Dependent Breast Cancer Cell Plasticity.*iScience* **23** , 101141, doi:https://doi.org/10.1016/j.isci.2020.101141 (2020).

59 Arnold, K. M., Opdenaker, L. M., Flynn, N. J., Appeah, D. K. & Sims-Mourtada, J. Radiation induces an inflammatory response that results in STAT3-dependent changes in cellular plasticity and radioresistance of breast cancer stem-like cells. *International Journal of Radiation Biology* **96** , 434-447, doi:10.1080/09553002.2020.1705423 (2020).

60 Ahmed, S. *et al.* IL-8 secreted by tumor associated macrophages contribute to lapatinib resistance in HER2-positive locally advanced breast cancer via activation of Src/STAT3/ERK1/2-mediated EGFR signaling. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1868** , 118995, doi:https://doi.org/10.1016/j.bbamcr.2021.118995 (2021).

61 Lu, L. *et al.* Activation of STAT3 and Bcl-2 and reduction of reactive oxygen species (ROS) promote radioresistance in breast cancer and overcome of radioresistance with niclosamide. *Oncogene***37** , 5292-5304, doi:10.1038/s41388-018-0340-y (2018).

62 Abreu de Oliveira, W. A. *et al.* Wnt/β-Catenin Inhibition Disrupts Carboplatin Resistance in Isogenic Models of Triple-Negative Breast Cancer. *Front Oncol* **11** , 705384, doi:10.3389/fonc.2021.705384 (2021).

63 Wan, H., Li, Z., Wang, H., Cai, F. & Wang, L. ST8SIA1 inhibition sensitizes triple negative breast cancer to chemotherapy via suppressing Wnt/β-catenin and FAK/Akt/mTOR. *Clin Transl Oncol* **23** , 902-910, doi:10.1007/s12094-020-02484-7 (2021).

64 Zhong, Z. & Virshup, D. M. Wnt Signaling and Drug Resistance in Cancer. *Mol Pharmacol* **97** , 72-89, doi:10.1124/mol.119.117978 (2020).

65 Nair, M. G. *et al.* miR-18a Mediates Immune Evasion in ER-Positive Breast Cancer through Wnt Signaling. *Cells***11** , doi:10.3390/cells11101672 (2022).

66 Kumar, S. *et al.* Dll1(+) quiescent tumor stem cells drive chemoresistance in breast cancer through NF-κB survival pathway.*Nat Commun* **12** , 432, doi:10.1038/s41467-020-20664-5 (2021).

67 BeLow, M. & Osipo, C. Notch Signaling in Breast Cancer: A Role in Drug Resistance. *Cells* **9** , doi:10.3390/cells9102204 (2020).

68 Wada, M., Horinaka, M., Yamazaki, T., Katoh, N. & Sakai, T. The dual RAF/MEK inhibitor CH5126766/RO5126766 may be a potential therapy for RAS-mutated tumor cells. *PLoS One* **9** , e113217, doi:10.1371/journal.pone.0113217 (2014).

69 Hoang, V. T. *et al.* Dual inhibition of MEK1/2 and MEK5 suppresses the EMT/migration axis in triple-negative breast cancer through FRA-1 regulation. *J Cell Biochem* **122** , 835-850, doi:10.1002/jcb.29916 (2021).

70 Mohan, C. D. *et al.* Brucein D modulates MAPK signaling cascade to exert multi-faceted anti-neoplastic actions against breast cancer cells. *Biochimie* **182** , 140-151, doi:10.1016/j.biochi.2021.01.009 (2021).

71 Cao, Y. *et al.* RNA-binding protein QKI suppresses breast cancer via RASA1/MAPK signaling pathway. *Ann Transl Med***9** , 104, doi:10.21037/atm-20-4859 (2021).

72 Li, H., Prever, L., Hirsch, E. & Gulluni, F. Targeting PI3K/AKT/mTOR Signaling Pathway in Breast Cancer. *Cancers* **13** , 3517 (2021).

73 Huang, J. *et al.* Gigantol inhibits proliferation and enhances DDP-induced apoptosis in breast-cancer cells by downregulating the PI3K/Akt/mTOR signaling pathway. *Life Sci* **274** , 119354, doi:10.1016/j.lfs.2021.119354 (2021).

74 Ma, Y. *et al.* Design, synthesis and biological evaluation of anilide (dicarboxylic acid) shikonin esters as antitumor agents through targeting PI3K/Akt/mTOR signaling pathway. *Bioorg Chem***111** , 104872, doi:10.1016/j.bioorg.2021.104872 (2021).

75 Zhu, K. *et al.* AKT inhibitor AZD5363 suppresses stemness and promotes anti-cancer activity of 3,3'-diindolylmethane in human breast cancer cells. *Toxicol Appl Pharmacol* **429** , 115700, doi:10.1016/j.taap.2021.115700 (2021).

76 Prajapati, K. S., Gupta, S. & Kumar, S. Targeting Breast Cancer-Derived Stem Cells by Dietary Phytochemicals: A Strategy for Cancer Prevention and Treatment. *Cancers (Basel)* **14** , doi:10.3390/cancers14122864 (2022).

77 Kohandel, Z., Farkhondeh, T., Aschner, M., Pourbagher-Shahri, A. M. & Samarghandian, S. STAT3 pathway as a molecular target for resveratrol in breast cancer treatment. *Cancer Cell Int* **21** , 468, doi:10.1186/s12935-021-02179-1 (2021).

78 Hosseinzadeh, A., Merikhian, P., Naseri, N., Eisavand, M. R. & Farahmand, L. MUC1 is a potential target to overcome trastuzumab resistance in breast cancer therapy. *Cancer Cell Int* **22** , 110, doi:10.1186/s12935-022-02523-z (2022).

79 Liu, Z. *et al.* Small molecule STAT3 inhibitor, 6Br-6a suppresses breast cancer growth in vitro and in vivo. *Biomedicine & Pharmacotherapy* **121** , 109502, doi:https://doi.org/10.1016/j.biopha.2019.109502 (2020).

80 Nguyen, N. M. & Cho, J. Hedgehog Pathway Inhibitors as Targeted Cancer Therapy and Strategies to Overcome Drug Resistance. *Int J Mol Sci* **23** , doi:10.3390/ijms23031733 (2022).

81 Tsao, A. N., Chuang, Y. S., Lin, Y. C., Su, Y. & Chao, T. C. Dinaciclib inhibits the stemness of two subtypes of human breast cancer cells by targeting the FoxM1 and Hedgehog signaling pathway. *Oncol Rep*

47 , doi:10.3892/or.2022.8316 (2022).

82 Brennan, K. & Clarke, R. B. Combining Notch inhibition with current therapies for breast cancer treatment. *Ther Adv Med Oncol***5** , 17-24, doi:10.1177/1758834012457437 (2013).

83 Yen, W. C. *et al.* Targeting Notch signaling with a Notch2/Notch3 antagonist (tarextumab) inhibits tumor growth and decreases tumor-initiating cell frequency. *Clin Cancer Res***21** , 2084-2095, doi:10.1158/1078-0432.Ccr-14-2808 (2015).

84 Jaiswal, A. *et al.* Therapeutic inhibition of USP9x-mediated Notch signaling in triple-negative breast cancer. *Proceedings of the National Academy of Sciences* **118** , e2101592118, doi:doi:10.1073/pnas.2101592118 (2021).

85 Torborg, S. R., Li, Z., Chan, J. E. & Tammela, T. Cellular and molecular mechanisms of plasticity in cancer. *Trends in Cancer***8** , 735-746, doi:https://doi.org/10.1016/j.trecan.2022.04.007 (2022).

86 Qin, S. *et al.* Emerging role of tumor cell plasticity in modifying therapeutic response. *Signal Transduction and Targeted Therapy* **5** , 228, doi:10.1038/s41392-020-00313-5 (2020).

87 Lüönd, F., Tiede, S. & Christofori, G. Breast cancer as an example of tumour heterogeneity and tumour cell plasticity during malignant progression. *British Journal of Cancer* **125** , 164-175, doi:10.1038/s41416-021-01328-7 (2021).

Tables:

Table1. Molecular pathways involved in cancer plasticity which impact drug resistance and possible therapeutic strategies.

Pathway	Related Drug Resistance	Potential Treatment	Inhibitor targeted
MAPK pathway	Chemoresistance Radiation resistance Enzyme therapy resistance	CH5126766 along with Eribulin Dual inhibitors Cobimetinib and Trametinib Brucein D (BD)	RAF/MEK inhibitor[68] MEK1/2 and MEK 5[69] p38 pathway[70]
PI3K/AKT/mTOR pathway	Chemotherapy Resistance Radiotherapy Resistance	Gigantol Anilide Shikonin ester- M9. AZD5363 alongside 3,3'-Diindoylmethane Dietary phytochemicals	Akt [73-75] Luminal A breast cancer cells [76]
STAT 3 pathway	Radiotherapy Resistance Chemotherapy resistance	Resveratrol Trastuzumab 6Br-6a	STAT3 [77-79]
Wnt/beta-catenin pathway	Chemoresistance Radioresistance Immunotherapy resistance	Tankyrase inhibitors such as XAV939 Introduction of truncated APC	Axin degradation[64] Lack of APC[64]
Hedgehog pathway	Chemoresistance Immunotherapy resistance	Smo inhibitors like Vismodegib,Sonidegib, Saridegib and Taladegib	Smo receptor[80,81]
Notch pathway	Chemoresistance Immunotherapy resistance	Gamma-secretase inhibitors such as Z-Leu-Leu-Nle-CHO Monoclonal antibodies like OMP-59R5	Gamma-secretase protein[82,84] Notch 2/ Notch 3 receptors[83]