Overcoming Resistance to Anti-Breast Cancer Drugs Targeting Kinases

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SUMMARY

Although there are no definitive cures, breast cancer may be treated through administration of drugs targeting specific protein kinases. Protein kinases are prominent factors in breast cancer, as they are essential for tumor cell proliferation and are highly traceable targets. These kinases are often targeted because of their ubiquity and capacity to become oncogenic. Patients can develop resistance to kinase inhibitors, increasing the risk of recurrence. As kinase inhibitors continue to be developed and used in clinical settings, there is an increased need to overcome resistance. The acquired resistance to kinases responsible for the proliferation of breast cancer cells has rendered many therapeutic strategies obsolete. A further understanding of the mechanisms allowing tumor cells to bypass targeted kinase inhibition can lead to improved treatments and the development of more effective drugs. This literature review provides a summary of the current state of research into therapeutics targeting protein kinases associated with breast cancer. Current protein kinase inhibitors for breast cancer will be discussed, as well as resistance mechanisms associated with these inhibitors. Mechanisms of resistance can be classified into five categories: 1) gene mutations, 2) alternative signalling and transcriptional changes, 3) alterations in transmembrane transportation, 4) off-target drug binding, and 5) cellular transitions. This review aims to summarize the current research in protein kinase inhibitor resistance mechanisms and development of novel therapeutics.

Received: 02/13/2019 **Accepted:** 04/08/2019 **Published:** 11/17/2019

Keywords: breast cancer, protein kinases, overcoming drug resistance, mutations, alternative signalling, transcriptional adaptation, cell transitions, transmembrane transportation

INTRODUCTION

EPIDEMIOLOGY

Breast cancer is the second leading cause of cancer death in women and presents a major public health concern, with a lifetime risk of developing this disease being approximately 11% for women. This cancer type is heterogeneous in pathology and current statistics suggest that worldwide incidence of breast cancer is on the rise (Tao, et al., 2015). Developing breast cancer is more likely in an older population, as a majority of the cases occur in women aged 50-69 (Statistics Canada, 2015). It is projected that by 2050, over 3 million new cases of breast cancer will be diagnosed annually (Tao, et al., 2015).

These statistics exhibit the magnitude of breast cancer incidence on global health and the increasingly pertinent need for treatment measures. While therapies have been successful in reversing the progression of breast cancer, many questions remain unanswered with regards to the underlying mechanisms that enable tumor cells to develop resistance to current therapeutics.

HALLMARKS OF CANCER

Cancer cells function differently compared to normal cells and the "hallmarks of cancer", developed by Hanahan and Weinberg in 2011, highlight the unique qualities associated with tumors. The hallmarks include uncontrollable cell proliferation, evading growth suppressors, replicative immortality, evading cell death, tissue invasion and metastasis, as well as inducing angiogenesis (Hanahan and Weinberg, 2011).

Cancer cells escape normal growth control by deregulating the checkpoint signals needed for division. Uncontrollable proliferation is promoted by the overexpression or hyperactivity of growth factor receptors and mutations (Sledge Jr and Miller, 2003). To acquire insensitivity to anti-growth signals, the negative feedback systems that regulate cell growth and proliferation fail to function properly. This indicates alterations in the expression of cyclins, cyclin-dependent kinases, and their inhibitors in order to evade growth suppressors (Hanahan and Weinberg, 2011). The shortening of telomeres is associated with senescence and promotes cell death, however, cancer cells bypass this process by expressing telomerase. Telomerase prevents the progressive shortening of telomeres and allows for the limitless replicative potential of cancer cells (Hanahan and Weinberg, 2011). Moreover, cancer cells with the capability of migrating from their origin to surrounding body tissues are considered to be malignant tumors. Tumors may secrete proteases that degrade surrounding tissues and downregulate the expression of proteins responsible for cell-cell adhesion (Sledge Jr and Miller, 2003; Miller, Goulet and Johnson, 2016). These processes allow for the invasion of cancer into other tissues within the human body, known as metastasis. The metastatic process is dependent on angiogenesis, which provides additional nourishment and aids in the spread of cancer (Hanahan and Weinberg, 2011). These six biological capabilities acquired by human tumors demonstrate the complexity and genetic diversity of cancer, slowing the advancement of breast cancer treatments.

PROTEIN KINASES

Kinases within the human body are essential in cell signalling as they play an integral role in regulating cell growth, survival, and apoptosis (Angus, Zawistowski and Johnson, 2017). Given their importance in cellular functions, the deregulation of kinases and uncontrolled kinase activity leads to the pathogenesis of various cancers. The abnormal activity of deregulated kinases drives tumor cell progression and gives them the critical features that make cancer such a difficult disease to cure (Angus, Zawistowski and Johnson, 2017).

Acquiring resistance to anti-cancer drugs prior to the administration of therapies is common in cancer cells, though the resistant species may not be dominant within the tumor. Treatments preferentially select for resistant cells as non-resistant species are susceptible to therapies. The mechanisms of tumor resistance to anti-cancer drugs can be classified into five categories: 1) mutations of the target protein, 2) signalling and transcriptional adaptation, 3) alterations in transmembrane transportation, 4) off-target drug binding, and 5) cellular transitions. Though many of these mechanisms may be applied to numerous types of cancers, these have all been documented within breast cancer and some mechanisms, such as cellular transitions, are more specific to breast cancer. This literature review provides an in-depth analysis of these five mechanisms of resistance and how kinases continue to be the driving force behind the progression of breast cancer through resistance to therapeutics. In analyzing the mechanisms of resistance, a further understanding of its relationship to the kinome can provide new strategies and novel pathways for kinase inhibitor therapy.

MECHANISMS OF KINASE INHIBITOR RESISTANCE

MUTATIONS OF TARGET PROTEINS

If a protein contains a point mutation within its coding region, the mutation will alter the protein's structure and modify its affinity for certain drugs and ligands. Since mutations can affect the binding of both the drug and ligand, either the drug's affinity must decrease or the affinity of the ligand must increase for resistance to arise. Mutations have the potential to confer resistance to a given drug. With respect to tyrosine kinases, relevant mutations can be classified according to their location within the protein: 1) gatekeeper mutations, 2) g-loop mutations, 3) a-loop mutations, and 4) αC helix mutations (Barouch-Bentov and Sauer, 2011).

Gatekeeper mutations involve the substitution of the amino acid which participates in the entry of

the drug into the active site of the protein. As such, mutations of the gatekeeper residue can readily lead to resistance, as seen in the proteins SCFR, EGFR, and ERBB2 (Barouch-Bentov and Sauer, 2011). An example of a gatekeeper mutation is that of the T790M mutation within EGFR (Yun, et al., 2008). In approximately 50% of cases in which resistance to inhibitors of EGFR is observed, the T790M mutation is present (Chen and Fu, 2011). The T790M mutation involves the substitution of a threonine, a polar amino acid, with methionine, a larger and nonpolar amino acid. This mutation has been found to result in an increased affinity for the ligand, adenosine triphosphate (ATP) (Yun, et al., 2008). Despite resistance introduced via the T790M mutation, it has been overcome through drugs that irreversibly bind to the C773 residue and drugs binding to other regions (Kwak, et al., 2005; Kobayashi, et al., 2005; Jia, et al., 2016). Similar to gatekeeper mutations, g-loop mutations may reduce drug efficacy against the target protein as the g-loop is involved in ATP binding (Barouch-Bentov, et al., 2009). Drugs targeting the g-loop often competitively inhibit ATP binding, indicating that alterations within the g-loop can impact drug effectiveness. Many mutations involving the g-loop either destabilize the inactive state or stabilize the active state, leading to hyperactivity (Cowan-Jacob, et al., 2007). Within the protein kinase BCR-ABL, the mutations G255K and G255V both lead to destabilization of the inactive state when bound to an inhibitor. Mutations G250E and Y253H also impact drug resistance, however, whether these stabilize the active state or destabilize the inactive state is more ambiguous. The a-loop and αC helix regions are involved in the transition between the active and inactive conformations and mutations within these regions may lead to changes in kinase activity (Liu and Gray, 2006; Ferguson, 2009; Fabbro, Cowan-Jacob, and Moebitz, 2015). Mutations within these regions tend to confer resistance to drugs by destabilizing the inactive conformation, increasing kinase activity (Barouch-Bentov and Sauer, 2011). A common mutation within the a-loop is L858R, which represents approximately 41% of oncogenic mutations in EGFR and significantly increases EGFR activity (Johnson, 2009). If a mutation causes the activity of a protein to increase, it is likely that the required dosage will increase. Mutations may also result in a protein that preferentially binds to its natural ligands rather than an inhibitory drug, leading to greater activity.

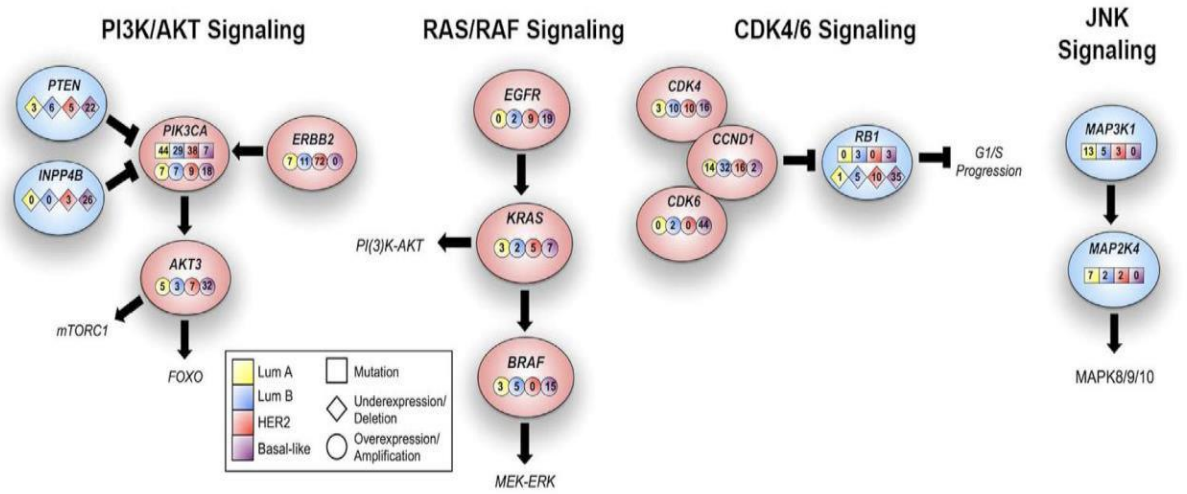

Figure 1: Known important signalling pathways involving kinases in breast cancer. Mutations in proteins either downstream or upstream of the target can potentially result in resistance to a given drug. If the mutant is downstream of the target protein, as is with PI3K and ERBB2, continual activation of the downstream mutant can result in drugs of the target being rendered ineffective. Similarly, if the mutant is upstream of the target, resistance to drugs can be attained by a mutant bypassing a protein in the signalling pathway, such as ERBB2 activating AKT3 rather than PI3K (Miller, Goulet and Johnson, 2016).

SIGNALLING AND TRANSCRIPTIONAL ADAPTATION

Cell signalling is essential for proliferation and growth, though some tumors have evolved alternative methods to maintain communication. Various treatments target cancer by inhibiting signalling pathways. To acquire resistance to treatments, protein kinases may activate pathways downstream of the protein target, resulting in insensitivity to inhibitors (Angus, Zawistowski and Johnson, 2017). Resistance through downstream activation allows signalling pathways to be activated even in the presence of an inhibitor targeting an upstream protein. For example, the protein phosphoinositide 3-kinase (PI3K) can be upregulated and confers resistance to inhibitors of ERBB2 (D'Amato, et al., 2015). This example may be especially concerning in the case of HER2-enriched breast cancer, which is characterized by greater ERBB2 expression (Iqbal and Iqbal, 2014). Continual activation of PI3K removes the need for activation by ERBB2, as PI3K is downstream of ERBB2 (Figure 1). In HER2-enriched breast cancer, ERBB2 is often targeted in therapies, yet PI3K upregulation may render such treatments ineffective. Rather than continual activation of a downstream protein, mutations in upstream proteins may result in resistance. Upstream proteins have the potential to undergo mutations, allowing them to bypass the target protein and altering the signalling pathway to omit the target protein. Bypasses subsequently cause treatments exploiting the target protein to be rendered ineffective and are often documented in HER2 enriched breast cancer (Miller, Goulet and Johnson, 2016).

Transcriptional adaptation shares similarities with the concept of signalling adaptation with a distinct mechanism. Adaptation via transcriptional modification involves the activation of signalling pathways and the upregulation of other proteins rather than a mutation in the pathway. This is how the amplification of the hepatocyte growth factor receptor (MET) confers resistance to gefitinib (Engelman and Settleman, 2008). The PI3K/AKT pathway is activated by ERBB2 or by EGFR through the RAS/RAF pathway. MET upregulation presents an alternative pathway by allowing ERBB3 to activate the PI3K/AKT pathway in lieu of ERBB2 and EGFR. Gefitinib, which inhibits EGFR, is no longer effective in this scenario since MET allows for ERBB3 to activate pathways previously activated by EGFR. Therapies would require inhibition of both EGFR and MET in order to remain effective.

TRANSMEMBRANE DRUG TRANSPORT

Many anti-cancer drugs target proteins within the cell rather than surface receptors. For a drug to be effective, it needs to first enter the cell, which may be mediated by transporter proteins. Transporter proteins surrounding the cell membrane selectively choose molecules to pass through the membrane (Bixby and Talpaz, 2009). With respect to drug influx, some drugs may enter the cell with the aid of transporters. The human organic cation transporter (HOCT) is an example of this process, as it allows the import of anti-cancer drugs such as imatinib (Andreev, et al., 2016; Thomas, et al., 2004). It has also been shown that inhibiting HOCT reduces intracellular drug concentrations, implicating a method of resistance. On the contrary, efflux transporters can force unwanted compounds out of the cell and decrease its concentration in the intracellular environment. Efflux pumps reduce the efficacy of a given drug and its cellular bioavailability. This mechanism can be seen in P-glycoprotein efflux pumps (PGP), which excludes drugs from the cell. It has been previously shown that inhibiting PGP restores the functionality of various drugs in otherwise resistant cells (Mahon, et al., 2000; Che, et al., 2002; Kotaki, et al., 2003). Overall, a decrease in the activity of influx pumps or an increase in efflux pump activity result in reduced intracellular drug concentrations and may lower the effectiveness of a drug.

OFF-TARGET DRUG BINDING

To avoid the therapeutic effects of anti-cancer drugs in the body, cancer cells can utilize different cellular proteins to interact with drugs. By providing molecules to interact and bind with the drug, cancer cells can effectively reduce drug availability. Certain basic drugs have been observed to interact with proteins, such as α1 acid glycoproteins which are found within blood plasma (Daub, Specht and Ullrich, 2004). When bound to α1 acid glycoproteins, the drug is sequestered and no longer able to work against its target, reducing cellular bioavailability and preventing the intended interaction with the target protein (Smith, et al., 2012). In breast cancer, α1 acid glycoproteins are capable of binding to drugs

like imatinib in blood plasma, reducing the treatment effects and cellular bioavailability. Also, the concentration of α1 acid glycoproteins is greater in patients with breast cancer, amplifying the effect of off-target drug binding (Smith, et al., 2012). This results in a greater dose required for the same effect, increasing the cost of treatment and potentially increasing side effects.

CELLULAR TRANSITIONS: EPITHELIAL TO MESENCHYMAL-LIKE CELLS

A method of gaining resistance to anti-cancer drugs is the transition of a breast cancer cell to a mesenchymal-like cell. By transitioning to a different cell type, the cancer cell acquires a new proteome which can lead to drug resistance, such as those inhibiting the PI3K/AKT pathway (Martz, et al., 2014). Transitional resistance may arise in any molecular subtype of breast cancer, though this tends to be more likely in claudin-low breast cancer due to the similarity of its proteome to that of mesenchymal cells (Miller, Goulet and Johnson, 2016). Claudin-low breast cancer involves expression of AXL and ZEB1, in addition to a lesser expression of e-cadherin and claudin-3. In a cell population which has transitioned to a more mesenchymal-like state, there may be an elevated expression of vimentin and n-cadherin. In transitional resistance, overexpression of Notch1, a protein that promotes the transition to mesenchymal cells, has been shown to provide resistance to inhibitors targeting the PI3K/AKT pathway (Fender, et al., 2015; Miller, Goulet and Johnson, 2016). Notch1 also reduces the expression of proteins responsible for intercellular adhesion, enabling cells to migrate through the extracellular matrix. Some mesenchymal transcriptional factors such as Twist, Snail, Slug, and ZEB2, control the expression of AXL and increase the likelihood of metastasis when upregulated. Overexpression of Notch1 has also been observed to cause resistance to EGFR and ERBB2 inhibitors. Due to the resistance conferred to ERBB2 inhibitors, transitional resistance may be particularly harmful in HER2-enriched breast cancer.

POTENTIAL STRATEGIES TO OVERCOME RESISTANCE

MUTATIONS OF TARGET PROTEINS

In a mutated protein resistant to therapeutics, the protein tends to become hyperactive or exhibit an increased preference for the protein to bind to the natural ligand rather than the drug (Kwak, et al., 2005). Mutations impacting drug binding can be overcome via rational drug design. Based upon the structure of effective anti-cancer drugs against the non-mutated protein, new drugs may be designed. Considering the changes in protein structure that come with mutations, such as the more common T790M mutation of EGFR, this can enable the development of novel drugs. By modifying the structure of a drug and accounting for sterics and functional group interactions, a drug specific to the mutant may be developed, though this would require screening patients for mutants in the event of resistance. Rather than developing new drugs to target the same region of a protein, attacking different portions of a protein may be useful. For example, the T790M mutation of EGFR has been overcome in the past by drugs that irreversibly bind to C773 (Kwak, et al., 2005; Kobayashi, et al., 2005). Alternatively, targeting downstream proteins may minimize the resistance conferred by an upstream mutation. A downstream target would eliminate the need for a drug to be effective against a particular mutant protein.

SIGNALLING ADAPTATION AND TRANSCRIPTIONAL ADAPTATION

Both upstream and downstream mutations may be treated through combination therapy. If both ERBB2 and PI3K were mutated to be constitutively active, a combination of drugs that inhibit ERBB2 and PI3K may be an effective treatment. A potential effective treatment could solely target PI3K since it is downstream of ERBB2, provided that ERBB2 does not operate through alternate pathways (Engelman and Settleman, 2008). Given a mutant ERBB2 that bypasses PI3K, therapy involving inhibitors of

both proteins may be necessary. With respect to transcriptional adaptation, combination therapy may also be required. In the case of MET upregulation, inhibitors targeting both MET and EGFR proved to be effective and both were required in treatment, as inhibiting only one allowed for the other to propagate signals (Engelman and Settleman, 2008).

TRANSMEMBRANE DRUG TRANSPORT

The presence of efflux and influx pumps provides numerous opportunities for overcoming resistance and reducing the side effects associated with drugs. Influx pumps may have therapeutic value, as activation of these pumps can potentially improve drug efficacy. As inhibition of the HOCT reduces cellular drug uptake, applying an activator of HOCT would increase drug uptake by cells (Thomas, et al., 2004). Similarly, as PGP can reduce intracellular drug concentrations, an inhibitor of PGP would lower net drug efflux and increase overall drug efficacy (Mahon, et al., 2000; Che, et al., 2002; Kotaki, et al., 2003). Though influx and efflux pumps may confer resistance to anti-cancer drugs, this also opens new avenues for treatments to increase cellular drug uptake, which may reduce side-effects. If a specific influx pump is overexpressed within the tumor, a treatment can be made more specific by targeting the influx pump, lowering the therapeutic dosage.

OFF-TARGET DRUG BINDING

Despite the presence of proteins that are able to sequester certain drugs, current treatments can still remain effective. Raising the dosage of a drug known to be sequestered may bypass resistance, though this could be dangerous for the patient receiving treatment. Conversely, the drug could be administered in tandem with a molecule that binds with sequestering proteins, like α1 acid glycoproteins, or those that reduce the concentration of sequestering proteins (Smith, et al., 2012). The anti-cancer drug tamoxifen is known to reduce α1 acid glycoprotein concentrations and may prove useful when given in conjunction with other anti-cancer drugs (Smith, et al., 2012). By reducing the propensity of α1 acid glycoproteins to drug binding, the sensitivity to the drug may increase and reduce the required dosage, potentially lowering the cost of treatment.

CELLULAR TRANSITIONS: EPITHELIAL TO MESENCHYMAL-LIKE CELLS

Regardless of the resistance conferred to inhibitors of various signalling pathways, including the PI3K/AKT, EGFR, and HER2 pathways, the transition to mesenchymal-like cells provides opportunities for treatments targeting pathways specific to mesenchymal cells. By transitioning, the cell attains a unique proteome, some of which may be targeted in the treatment of cancer (Martz, et al., 2014). The Slug protein is thought to have anti-apoptotic activity (Inukai, et al., 1999). Targeting the Slug protein for inhibition could potentially induce apoptosis, providing a viable treatment for mesenchymal-like cancer cells.

CONCLUSION

The emergence of protein kinase inhibitor resistance causes many drawbacks of breast cancer treatment. It undoubtedly poses challenges for current successful anti-cancer therapies as breast cancer biology remains a diverse field. Protein kinases are common drug targets for cancer treatment as they are important for tumor cell proliferation and survival. However, the six deemed biological traits of cancer and its ability to acquire resistance to kinase inhibitors through protein mutations, signalling and transcriptional adaptation, transmembrane drug transport, off-target drug binding, and transitional resistance make cancer a challenging disease to cure. Nevertheless, mutations may be overcome thr-ough rational drug design and targeting conserved areas of the target protein. Signalling and transcriptional adaptation could be bypassed by targeting downstream proteins or alternative pathways, as well as combination therapies. Transmembrane drug transportation offers a method to enhance cellular bioavailability and reduce the required therapeutic dose. On the other hand, off-target drug binding increases the therapeutic dose, yet lowering the levels of sequestering proteins can help remediate this. Transitional resistance entails a modified proteome and kinome, providing other pathways that may be exploited in treating breast cancer. Gaining a further understanding allows researchers to continually improve the efficacy of anti-cancer drugs and identify novel target molecules to overcome kinase inhibitor resistance in prospective clinical studies. The overarching goal of this review is to distinguish the key pathways

driving resistance for breast cancer cells in order to aid in the design of novel therapies.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr. John Hassell for his helpful advice on the various topics examined in this review paper.

AUTHOR CONTRIBUTIONS

This collaborative piece was divided between M.C. and J.D.. M.C. wrote the introduction, mechanisms of kinase inhibitor resistance and conclusion. J.D. wrote the abstract, mechanisms of kinase inhibitor resistance and potential strategies to overcome resistance.

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