

# PTEN as a Candidate Molecular Target that Links MVA Pathway and Anti-HER2 Therapy Resistance

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## ABSTRACT

Drug resistance remains a major problem hampering the success of targeted treatment in cancer therapy. Despite the promising future of targeted therapy, the occurrence of drug resistance leading to poor therapeutic response has been recorded in a large number of patients. The underlying mechanism however is not conclusive. Cholesterol has been associated with the development of breast cancer and its link to drug resistance in this type of cancer could be a new branch of study. Targeting cholesterol biosynthesis for cancer therapy via the mevalonate (MVA) pathway is currently demonstrated as an attractive approach for overcoming treatment resistance in HER2+ breast cancer. Here, we discuss the overview of MVA pathway components in breast cancer treatment and resistance as well as the potential use of MVA pathway inhibitors in breast cancer therapy as a new approach to the problem. We also highlight the role of PTEN deficiency, a well-known resistance mechanism in HER2+ breast cancer cells, as a possible new target of the HMGCR inhibition that could be exploited to reverse anti-HER2 resistance.

## Keywords

cholesterol, therapy resistance, breast cancer, HER2, PTEN

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## INTRODUCTION

Globally, millions of deaths linked to cancer have been reported each year and the figure is expected to increase due to lifestyle factors.<sup>1,2</sup> Breast cancer is the most common cancer among females. In 2018, the number of new breast cancer cases among females worldwide was 47,500.<sup>3</sup> 7,593 breast cancer cases were registered in Malaysia 2018, accounting for 32.7% of female cancer cases in the same year.<sup>3</sup> A study reported that Malaysian women with breast cancer have poor survival rates and usually presented themselves at a later stage of cancer.<sup>4</sup> HER2 positive (HER2+) breast cancer is a well-known breast cancer molecular subtype that makes up a significant proportion, about 20-25% of breast cancer cases.<sup>5</sup> This molecular subtype of breast cancer arises from overexpression of Human Epidermal Growth Factor Receptor-2 (HER2).

Protein tensin homolog deleted on chromosome 10 (PTEN) is an important regulator of cell proliferation and survival. Several findings highlight the significant roles of PTEN in maintaining normal cellular activities.<sup>6,7</sup> In the

cytoplasm, PTEN acts by dephosphorylating PI3K/AKT pathway components.<sup>7</sup> This action by PTEN thus affects a wide range of cellular activities downstream to the pathway including cell cycle progression, apoptosis induction, and angiogenesis.<sup>6,7</sup> Mutation of the PTEN gene has been implicated in many diseases such as rheumatoid arthritis, chronic obstructive pulmonary disease, and pulmonary fibrosis.<sup>6</sup> Development of many types of cancer has also been linked to PTEN loss.<sup>6,7</sup> In the nucleus, the tumour-suppressing activity of PTEN includes maintenance of genomic integrity, DNA repair, and breakdown of oncoprotein.<sup>7</sup> Loss of PTEN function by germline mutation increased the risk of breast cancer due to Cowden Syndrome. This condition predisposes an individual to breast cancer up to 85% risk.<sup>7,8</sup> Reduced level of PTEN has a significant relation with tumour grade of breast cancer.<sup>7</sup> Furthermore, the report also mentioned that complete loss of PTEN is more frequently found in metastatic breast cancer compared to primary breast cancer. PTEN has also been shown to play an important role in the development of HER2+ breast cancer, with

evidence that PTEN loss enhances the proliferation and metastasis of HER2+ tumour cells in a mouse model.<sup>9</sup>

Association between cholesterol and cancer progression remains controversial. Studies have found that cholesterol and cholesterol regulator levels were reportedly higher in breast cancer.<sup>10</sup> Several studies highlighted that cholesterol levels could have different effects on breast cancer, depending on patients' age.<sup>11,12</sup> Among premenopausal women, a lower risk of breast cancer was found with high body mass index (BMI) cases.<sup>12</sup> However, this effect was the opposite with patients of postmenopausal age.<sup>12</sup> Breast cancer among obese premenopausal women was also linked to the more aggressive subtype TNBC.<sup>13,14</sup> In the postmenopausal women group, high BMI was associated with an increased risk of development of estrogen and progesterone receptor-positive breast cancer subtypes.<sup>15</sup> Overall, there was generally a 30% increased risk of breast cancer among obese women in the age group, and with a 5 kg/m<sup>2</sup> increase in BMI.<sup>16,17</sup> Similar outcome was also seen with breast cancer prognosis. Shorter disease-free survival and higher breast cancer mortality rate were found in obese patients across all age groups.<sup>18,19</sup> Association between higher BMI was also observed with larger tumor size, presence of lymph node involvement, and lower overall survival of breast cancer patients.<sup>20–22</sup> Therefore, it was concluded that cholesterol correlates with cancer progression. Further studies will be required to confirm the causative relationship between cholesterol and cancer progression.

Cholesterol is a vital molecule produced in the endoplasmic reticulum of cells that carry out important functions in our body such as cell structure components and intracellular trafficking.<sup>23</sup> The mevalonate pathway (MVA pathway) is the biosynthetic pathway responsible for producing cholesterol using Acetyl-Coenzyme A as the starting molecule.<sup>24</sup> The rate-limiting step of the complex pathway is controlled by an enzyme called 3-hydroxy-3-methylglutaryl Coenzyme A Reductase (HMGCR) which reduces HMG Coenzyme A into mevalonate.<sup>24</sup> Given the importance of HMGCR in the pathway, targeting this enzyme is potentially useful in studying the relationship between cholesterol production and cancer.<sup>10</sup> This will further provide insights on potential treatments of cancer

using MVA pathway inhibitors. This important association has not been studied in HER2+ breast cancer.

Statins are a class of drugs commonly used for the treatment of dyslipidaemia, another major health problem affecting the world population.<sup>25</sup> Statins function as competitive inhibitors of HMGCR in the rate-limiting step of sterol biosynthesis.<sup>25</sup> Besides dyslipidaemia, statins have also shown potential use in the treatment of cancer.<sup>25</sup> In breast cancer, mevalonate blockers showed potential in overcoming trastuzumab resistance, enhancing the effect of trastuzumab on HER2+ cell lines including SKBR3.<sup>26</sup>

Furthermore, researchers have also found that Epidermal Growth Factor Receptors (EGFR) are involved in upregulating enzymes in the MVA pathway including HMGCR.<sup>27</sup> This association between MVA pathway components in the cholesterol biosynthesis and breast cancer biomarker family could be an indicator of certain roles played by cholesterol in this type of cancer. Here, we discuss the overview of MVA pathway components in breast cancer treatment and resistance as well as the potential use of MVA pathway inhibitors in breast cancer therapy as a new approach to the problem.

## **The Scenario of HER2+ Breast Cancer Incidence Worldwide and Malaysia**

The HER2 receptor is a member of a family of tyrosine kinase receptors which also include HER1, HER3, and HER4. This receptor family carries out important functions involving cell proliferation, differentiation, and survival.<sup>28</sup> The receptors are activated by ligand binding at the outer domain, followed by dimerization between the receptors and phosphorylation of the cytoplasmic domain to activate downstream pathways.<sup>28</sup> HER2 has a distinctive characteristic of being activated without ligand binding and is preferred as a dimerisation partner by the other receptors.<sup>28</sup> Therefore, overexpression of HER2 receptors causes constitutive activation of their downstream pathways such as phosphatidylinositol 3-kinase (PI3K pathway) and mitogen-activated protein kinase pathway (MAPK pathway) (Figure 1).<sup>28,29</sup> Since these pathways are responsible for cell division and survival, continuous activation will ultimately result in

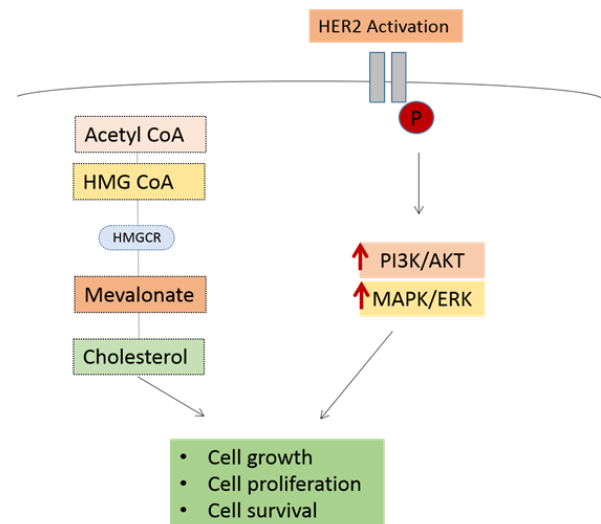
carcinogenesis.<sup>28</sup>

Globally, HER2+ breast cancer accounts for 20-25% of breast cancer cases in 2019.<sup>5</sup> Overexpression of HER2 receptors in breast cancer cells occurs when the cancer cells carry amplified HER2 genes of up to 25 to 50 copies to produce a maximum of 100 times more HER2 protein than normal cells.<sup>28</sup> As a result, 2 million HER2 receptors will be expressed on the cell surfaces.<sup>28</sup> In local clinical practice, HER2 status is routinely determined by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH).<sup>28,32</sup> IHC staining is used for the detection of HER2 protein expression whereas FISH is used to determine gene amplification.<sup>28,29</sup> Breast cancer specimens are initially tested using IHC staining for HER2 expression based on membrane staining.<sup>28,32</sup> Intensity and positive cell fraction are taken into account for the scoring. The scores are recorded on a scale of 0 to 3+ whereby a score of 0 to 1+ is considered negative, a score of 2+ as equivocal, and a score of 3+ as positive.<sup>32</sup> Specimens with equivocal scores will further be tested using FISH to determine the positive or negative status of HER2.<sup>28,29</sup>

HER2+ breast cancer is associated with poorly differentiated tumor cell characteristics and a lower overall survival rate compared to other subtypes.<sup>33</sup> In 2014, HER2+ breast cancer was recorded in 28% of all breast cancer cases in Malaysia.<sup>34</sup> Several treatments are available for breast cancer patients in local hospitals such as surgery, chemotherapy, radiotherapy, hormonal therapy, and targeted therapy.<sup>35</sup> In the local setting, trastuzumab targeted therapy is used as adjuvant treatment for HER2+ breast cancer but is only accessible to 19% of the patients.<sup>36</sup>

### Targeted Therapy for HER2+ Breast Cancer

Due to the limitations of target specificity in conventional treatments, targeted treatment becomes more favourable. Targeted treatment affects only cancer cells while healthy cells are uninterrupted. HER2 overexpression in breast cancer leads to continuous activation of many downstream pathways, increasing the mortality rate among patients.<sup>29</sup> Advancement in the medical field has seen various



**Figure 1.** HER2 activation triggers the activation of downstream PI3K/AKT & MAPK/ERK leading to cell growth, cell proliferation, and cell survival.<sup>14</sup> In addition, the cholesterol biosynthesis regulator, HMG-CoA reductase (HMGCR) plays a role in promoting breast cancer development and progression.<sup>30,31</sup>

targeted therapy designed for HER2+ breast cancer in order to solve the problem.

Trastuzumab is one of the first targeted drug treatments approved by the Food and Drugs Administration (FDA). This drug is a recombinant monoclonal antibody that targets extracellular domains of the HER2 receptors.<sup>37</sup> Another targeted therapy for HER2+ breast cancer patients is pertuzumab, a monoclonal antibody that blocks HER2/HER3 heterodimerization. This drug is effective when used alone, but a previous study found that there is also synergistic potential between pertuzumab and trastuzumab.<sup>38</sup> TD-M1 (trastuzumab emtansine) is another approved drug for HER2+ breast cancer treatment that consists of antibodies conjugated with cytotoxic drugs that specifically target HER2+ cancer cells, increasing treatment efficiency due to the delivery of the drug into the cells.<sup>39</sup>

Lapatinib is a reversible tyrosine kinase inhibitor of EGFR/HER2 that is capable of blocking both EGFR and HER2. Lapatinib is an inhibitor of PI3K/Akt and MAPK/Erk1/2 pathways.<sup>40</sup> Researchers found a synergistic effect of combined treatment of lapatinib and capecitabine, which gives a better outcome for patients with prior trastuzumab exposure.<sup>41</sup> Another study has demonstrated that lapatinib could act against HER2+ cancer cells by inducing apoptotic activity.<sup>42</sup> Furthermore,

a combination of trastuzumab and lapatinib in treatment also gives better results compared to monotherapy of either drug alone.<sup>43</sup> Afatinib and neratinib are two second-generation tyrosine kinase inhibitors that irreversibly inhibit more than one HER family member, both inhibitors have shown their efficacy in clinical trials either as monotherapy or in combination.<sup>44</sup>

Combination of anti-HER2 therapy by covalently binding to the receptors and preventing autophosphorylation and endocrine therapy such as letrozole and anastrozole is also one of the targeted therapies, particularly in postmenopausal women. The combinations of these endocrine treatments with trastuzumab or lapatinib were associated with significantly improved progression-free survival rates.<sup>40</sup> mTOR inhibitors are another type of targeted therapy against HER2- amplified breast cancer, which may even be beneficial against trastuzumab resistance, as demonstrated by a combination of taxane and trastuzumab in the presence of everolimus, which results in significant improvement of survival rates.<sup>40</sup>

### The Action of Trastuzumab Therapy

The FDA defined trastuzumab as a recombinant humanised monoclonal antibody, directed against extracellular domain IV of HER2 receptors.<sup>38</sup> This drug act specifically against HER2+ cells by various mechanisms such as blocking HER2 dimerization, HER2 internalisation by endocytosis, and triggering antibody-dependent cellular cytotoxicity (ADCC).<sup>5,41,45</sup> At the intracellular environment, trastuzumab triggers HER2 endocytosis by high-affinity binding to the receptor and subsequently forming a crosslinked complex with HER2.<sup>46</sup> Ultimately, the receptor is internalised by the cell.<sup>46</sup> In addition, trastuzumab blocks EGF receptor dimerisation in HER2-overexpressing cells, preventing downstream signaling of Akt/PI3K and MAPK thus affecting the inhibition of cell proliferation, growth, and survival (Figure 2).<sup>29,47</sup> Following trastuzumab binding, PTEN becomes activated and further increases the anti-tumour effect of trastuzumab.<sup>29</sup> Another mechanism of action of trastuzumab is HER2 endocytosis which leads to HER2 downregulation.<sup>45</sup> ADCC is an example of an extracellular mechanism of trastuzumab action, mainly

involving natural killer cells (NK cells).<sup>5,41,45</sup> Trastuzumab binding to HER2- amplified cancer cells attract NK cells to bind to trastuzumab and lyse the tumor cells.<sup>45</sup>

### Molecular Events Related to Trastuzumab Resistance

The mechanisms of trastuzumab resistance involve the steric effect, whereby trastuzumab binding to HER2 is blocked due to proteolysis of the receptors by metalloprotease ADAM10 (a disintegrin and metalloproteinase domain-containing protein 10) or by post-translational modification.<sup>45</sup> In this process, HER2 receptors become mutated, forming truncated p95HER2 isoform with no extracellular domain (ECD).<sup>45</sup>

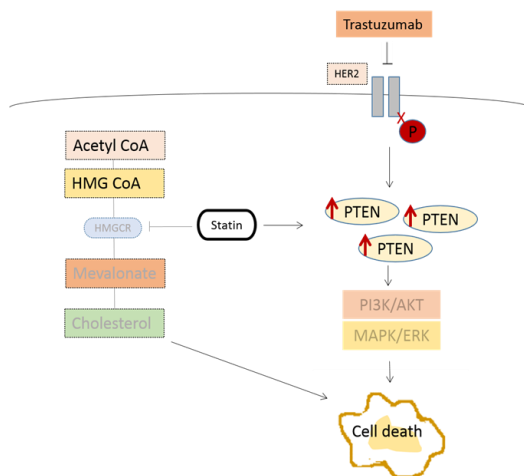
Trastuzumab resistance in cells can also take place by epitope masking with MUC 4 (Mucin 4) and CD44 (Cluster of differentiation 44) complexes. MUC 4 is a membrane-associated glycoprotein that consists of several highly glycosylated proteins. These proteins form protective barriers to mammary epithelial cells and also myoepithelial cells. The protective barriers inhibit the recognition of the cancer cells by the immune system, promoting tumor progression.<sup>38</sup> Epitope masking with CD44 may also be a possible mechanism of trastuzumab resistance. CD44 is a transmembrane receptor for hyaluronan and the binding of the hyaluronan polymer to CD44 may activate the PI3K/Akt activation that is responsible for tumor cell growth.<sup>38</sup>

Trastuzumab resistance may also occur as a result of intrinsic alterations- interference of PTEN, increased PI3K/Akt activity, and the modulation of p27<sup>KAI1</sup>.<sup>45</sup> PTEN inhibits the activation of the PI3K pathway, one of the downstream signaling pathways that contribute to tumor progression, which is a target for trastuzumab action. Reduction in PTEN level increases phosphorylation of PI3K/Akt, thus overcoming the effect of trastuzumab-mediated growth inhibition in HER2- amplified cancer cells.<sup>45</sup> Downregulation of PTEN controls sensitivity to trastuzumab treatment *in vitro*, *in vivo*, and clinical subjects.<sup>50,51</sup> HER2+ breast cancer patients with reduced PTEN expression showed poor response to trastuzumab treatment compared to patients with normal PTEN levels.<sup>50</sup>

Immunohistochemistry study revealed that in the majority of patients with high PTEN levels, 65.8% showed sensitivity to trastuzumab, whereas only 11.1% of patients with low PTEN were responsive to the same treatment.<sup>50</sup> Moreover, this observation was only specifically true for trastuzumab treatment. Both groups of patients showed a similar response when treated with other drugs such as taxane.<sup>50</sup> In the xenograft model, PTEN deficient tumours were more resistant to trastuzumab compared to those with normal PTEN levels as measured by mean tumour volume.<sup>50</sup> Following trastuzumab treatment, mouse xenograft models treated with PTEN antisense showed increasing tumour volume, reaching 600 mm<sup>3</sup> during week 4 of the treatment whereas those with normal PTEN expression produced tumour volume of 400 mm<sup>3</sup> after the same period of exposure to trastuzumab.<sup>50</sup> Knockdown of PTEN gene in HER2+ breast cancer cell BT474 also led to responsiveness to trastuzumab treatment.<sup>51</sup> According to DNA microarray analysis, genomic extracts of trastuzumab-resistant cells were significantly enriched with PTEN shRNA after 4 weeks of treatment with trastuzumab.<sup>51</sup>

the PI3K/Akt pathway.<sup>45</sup> Growth inhibitory properties of trastuzumab depend on the cyclin-dependent kinase-inhibiting protein p27<sup>KAIPI</sup> because this inhibitor is effective in decreasing cell proliferation and enhancing apoptosis, whereas decreased expression of this protein can lead to trastuzumab resistance.<sup>45</sup>

Resistance to trastuzumab also occurs by increased signaling from other EGFR family members. Despite the known effectiveness of trastuzumab action against HER2 in breast cancer, dimerisation among other receptors in the family can still activate downstream pathways although in the presence of trastuzumab.<sup>45</sup> Increased signaling from other receptors such as IGF-1R (Insulin-Like Growth Factor-1 Receptor) overexpression can also cause trastuzumab resistance. IGF-1R is a transmembrane tyrosine kinase receptor that shares a common downstream signaling pathway with HER2. Therefore, IGF-1R downstream effectors promote cell proliferation and metastasis. IGF-1R that interacts with HER2 can mediate trastuzumab resistance. This binding may involve in PI3K/Akt pathway and lead to the degradation of p27.<sup>2</sup>



**Figure 2.** Trastuzumab blocks HER2 activation and reduces the signaling PI3K/AKT & MAPK/ERK resulting in inhibition of cell growth, while PTEN is activated by trastuzumab action.<sup>32</sup> In addition, HMGCR inhibition via statin treatment also downregulates HER2 expression and induces cell death in HER2+ breast cancer cells.<sup>48</sup> Treatment with statin increases PTEN levels by interfering with NF $\kappa$ B transcriptional activity, reversing NF $\kappa$ B-mediated PTEN repression.<sup>49</sup>

## Cholesterol Synthesis as a Risk Factor for Breast Cancer

Over the past three decades, the global obesity rate has continuously increased, resulting in nearly 10% of the world population being obese.<sup>52</sup> In the local setting, Malaysia became the country with the highest prevalence of obesity and overweight in Southeast Asia, recorded at 46.3%.<sup>53</sup> Obesity is a condition defined by the accumulation of excessive body fat which leads to impairment of physical functions and an increase in the risks of certain illnesses.<sup>53</sup> Recent studies suggested a link between obesity and cancer-related death, whereby mortality rate was also the lowest among those with a normal range of BMI.<sup>54</sup> Studies have also found that obesity has a positive correlation to increased breast cancer risk, 2% for each 5 kg/m<sup>2</sup> increase in BMI.<sup>55</sup> Obesity is also very closely related to cholesterol level which accumulates in our body through food intake or naturally produced by the MVA pathway.

PI3KR1 and PI3KCA genes encode the subunit of p85 of the PI3K/Akt pathway and subunit of p100 of the PI3K pathway. Both genes are related to trastuzumab resistance by increasing the activity of the PI3K/Akt pathway. These genes are frequently mutated and overexpressed in breast cancer cases, resulting in constitutive activation of

Under normal circumstances, cholesterol and free fatty

acid (FFA) are processed together by enterocytes in the intestine into chylomicrons.<sup>56</sup> This molecule is then secreted into lymphatic vessels before entering circulation.<sup>57</sup> Chylomicrons and VLDL (Very Low-Density Lipoprotein) then deliver FFA to the heart, skeletal muscle, and adipose tissue for energy production and storage function.<sup>57</sup> The release of FFA from VLDL and chylomicrons is primarily mediated by lipoprotein lipase (LPL), a process known as lipolysis.<sup>57</sup> This enzyme is in turn controlled by insulin which also facilitates the uptake of FFA by adipocytes.<sup>58</sup> The FFA taken up by cells are later re-assembled into fat in the cytoplasm whereas chylomicrons remnants and VLDL are transported back to the liver.<sup>58</sup> During energy starvation, FFA will be released again from the cells, with a reduced level of insulin.<sup>57</sup> Due to these functions of insulin, it is suggested that this hormone does play a major role in lipoprotein metabolism. One of the cellular events that indicate dyslipidaemia in obesity is when triglycerides accumulate in the liver, leading to increased production of VLDL by the liver.<sup>59</sup> Since VLDL competes with chylomicrons for FFA uptake by myocytes and adipocytes, a high level of VLDL could impair the lipolysis which causes a high level of fat and cholesterol to be transported back into the liver.<sup>59</sup> Alternatively, VLDL and chylomicrons may be transported to tissues for the removal of cholesterol, a process that usually takes place in the liver. In other tissues, cholesterol may not be processed efficiently, leading to cholesterol accumulation in the tissues.<sup>59</sup>

In breast cancer, it was demonstrated that cholesterol accelerated tumor growth, increased aggressiveness of the tumour, and promoted angiogenesis.<sup>60</sup> Furthermore, adipocytes in vitro culture was found to promote breast cancer cell proliferation.<sup>60</sup> Studies have also suggested that tumor cells tend to overexpress Scavenger Receptor Type B1 (SR-B1) which is responsible for cellular uptake of cholesterol, a receptor that was suspected to cause cancer aggressiveness and poor survival among breast cancer patients.<sup>61</sup>

### **MVA Pathway and Its Components**

The MVA pathway is an important metabolic pathway that produces the precursor for isoprenoids in most

eukaryotes, archaea, and several eubacteria with Acetyl Coenzyme A as a precursor.<sup>24</sup> Examples of important enzymes at the start of this pathway are acetoacetyl CoA thiolase, HMG-CoA synthase, HMG-CoA reductase, phosphomevalonate kinase, and mevalonate diphosphate decarboxylase. These enzymes react in the reaction sequence to produce the precursor of isoprenoids, farnesyl pyrophosphate (FPP).<sup>24</sup> After the production of FPP, the MVA pathway diverges into different reactions to ultimately yield the many types of isoprenoids such as coenzyme Q, cholesterol, isopentenyl adenosine, and dolichol.<sup>24</sup> These metabolites play vital roles in promoting membrane association, tRNA (transfer RNA) modification, protein glycosylation, and mitochondria, forming the electron transport chain.<sup>23</sup>

A recent study reported that the MVA pathway is such a vital metabolic process that the inhibition of this pathway in cancer cells affects the epigenetic activity of the cells. MVA pathway inhibition reduces the level of NADPH (nicotinamide adenine dinucleotide phosphate), a product of glycolysis and fatty acid oxidation that regulates DNA repair genes in the cells.<sup>62</sup> Another importance of the MVA pathway is that the precursors and metabolites of cholesterol also function as signaling molecules in the cells.<sup>24</sup> This biosynthetic pathway of cholesterol production has also been discovered to play a significant role in the replication of mammalian cells.<sup>23,24</sup>

### **HMGCR, the MVA Pathway Precursor Associated with Breast Cancer Progression**

The cholesterol biosynthesis regulator, HMG-CoA reductase (HMGCR) catalyses the conversion of HMG-Co A to mevalonic acid, a crucial step in the biosynthesis of cholesterol.<sup>63</sup> HMGCR expands beyond its direct role in cholesterol synthesis following the discovery that HMGCR promotes breast cancer development and progression (Figure 1).<sup>30,31</sup> In the clinical setting, high levels of expression of cholesterol biosynthesis genes that include HMGCR were associated with shorter recurrence-free survival (RFS) and overall survival in estrogen receptor-positive (ER+) breast cancer.<sup>64</sup>

An evaluation from the Malmö Diet and Cancer Study

breast cancer cohort demonstrated that HMGCR moderate/strong expression was associated with worse prognosis such as higher histological grade, high Ki67, and ER negativity.<sup>65</sup>

Another study found that HMGCR staining was associated with triple-negative breast cancer (TNBC) in Korean patients.<sup>66</sup> Additionally, ER+ patients with positive expression of HMGCR were shown to have an improved response to tamoxifen treatment, indicating the role of HMGCR as a predictive biomarker for tamoxifen sensitivity in breast cancer.<sup>67</sup>

In HER2-overexpressing cell lines, the upregulation of HMGCR was found to be modulated by HER2 overexpression.<sup>26</sup> HER2-overexpressing cell lines showed high expression of HMGCR whereas HER2- negative cells did not exhibit a similar reaction.<sup>26</sup> In addition, HMGCR inhibition via statin treatment also downregulates HER2 expression and induces cell death in HER2+ breast cancer cells (Figure 2).<sup>48</sup> Both studies have shown that HMGCR expression plays a role in HER2+ breast cancer progression, however, its prognostic or predictive role should be further investigated in HER2+ breast cancer patients.

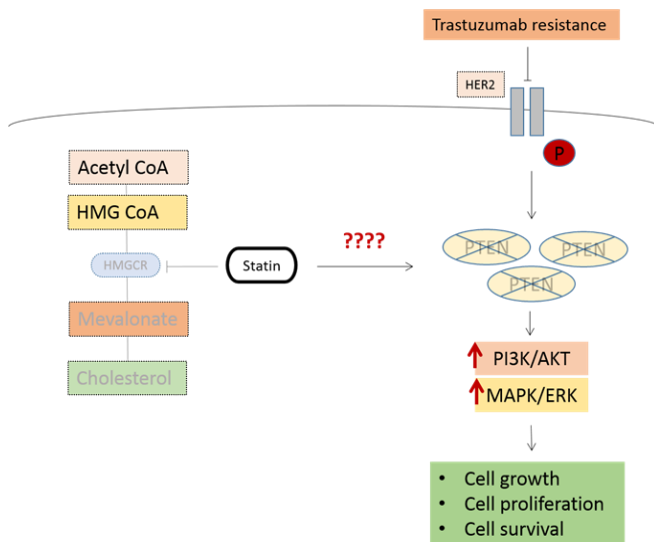
### **The Implication of Anti-MVA Pathway in Breast Cancer Treatment**

Besides the conventional use of anti-HMGCR or the so-called “statin” in treating hypercholesterolaemia, the drug has also been studied in breast cancer.<sup>25</sup> Combined statin and tamoxifen treatment inhibited cell growth and induced apoptosis in both *in vitro* and *in vivo* tamoxifen-resistant ER+ breast cancer models.<sup>25</sup> It was also found that treatment with statin prevented the growth and survival of TNBC cells in mice.<sup>49</sup> In addition, HMGCR inhibition also downregulated HER2 expression and induced cell death in HER2+ breast cancer cells (Figure 2).<sup>48</sup> A recent study has demonstrated that the MVA inhibitors, including statin, enhanced anti-HER2 response for the MVA-dependent drug-resistant cells.<sup>26</sup> In a similar study, the SYMPHONY clinical trial has shown that adding a statin to a dual anti-HER2 regimen re-sensitised tumours to anti-HER2 treatment.<sup>26</sup>

### **Molecular Mechanisms Underlying the Link between MVA Pathway and anti-HER2 Therapy Resistance**

PTEN upregulation, in general, has been shown to decrease breast cancer cell growth and increase apoptosis by suppressing Akt activation (Figure 2). PTEN levels, on the other hand, were shown to be elevated in breast cancer cells treated with a statin, indicating a link between PTEN and the anti-MVA pathway in breast samples.<sup>49</sup> Treatment with statin also reportedly increased PTEN levels by interfering with NF $\kappa$ B transcriptional activity, reversing NF $\kappa$ B-mediated PTEN repression in breast cancer.<sup>49</sup> PTEN also played an important part in anti-HER2 treatment since decreased PTEN levels were linked to trastuzumab resistance in HER2+ breast cancer (Figure 3).<sup>45</sup> However, the potential link between the MVA pathway and the effect of its inhibitor on PTEN downregulation concerning trastuzumab resistance should be looked into further.

Recently, a link between the MVA pathway and anti-HER2 therapy resistance was found through the linking of YAP/TAZ (Yes-associated protein/Transcriptional Coactivator with PDZ-binding motif), mTORC1 (mechanistic target of rapamycin complex 1), and Survivin.<sup>26</sup> Trastuzumab resistant cells' proliferation was significantly suppressed when YAP/TAZ was downregulated.<sup>26</sup> Another result found that the overexpression of YAP/TAZ, on the other hand, limits cell susceptibility to trastuzumab treatment.<sup>26</sup> Furthermore, MVA pathway activity was shown to be increased in trastuzumab-resistant cells, and inhibition of this pathway resulted in YAP/TAZ and mTORC1 downregulation.<sup>26</sup> This study also shows Survivin's potential function as a molecular junction that might interact with either the YAP/TAZ pathway, the mTORC1 pathway, or the MVA pathway to promote the survival of trastuzumab-resistant cells. However, the possible relationship between the aforementioned associations and PTEN downregulation in relation to trastuzumab resistance is yet unknown. Meanwhile, the YAP/TAZ and mTOR pathways have been linked to PTEN expression in TNBC cells lacking HER2.<sup>68</sup> YAP was shown in this study to activate mTOR through downregulating PTEN.<sup>68</sup> Based on the available findings,



**Figure 3.** Reduction in PTEN level increases phosphorylation of PI3K/ Akt, which prevents the effect of trastuzumab-mediated growth inhibition in HER2+ cancer cells.<sup>45</sup> The possible association of the MVA pathway, its inhibitor, and PTEN in relation to trastuzumab resistance should be further explored.

PTEN, YAP/TAZ-mTOR, as well as Survivin are crucial molecules to consider when describing the molecular mechanisms underlying the relationship between the MVA pathway and anti-HER2 treatment resistance.

## CONCLUSION

Targeted therapy for breast cancer remains an important discovery that could open new possibilities in improving the chances of survival of the patients suffering from the disease. However, cases of drug resistance can disrupt the beneficial effect of the treatments. Future work in understanding the mechanisms of drug resistance is important in solving the problem. Since cholesterol has shown remarkable involvement in cancer progression and tumorigenesis, targeting cholesterol biosynthesis for cancer therapy via the MVA pathway is currently demonstrated as an attractive approach for overcoming treatment resistance in HER2+ breast cancer. HMGCR inhibitor, statin may circumvent resistance to anti-HER2 therapy warranting further clinical investigation. Given the association between PTEN and YAP/TAZ-mTOR-survivin, the possible association of anti-HMGCR (statin) and PTEN in trastuzumab resistance could serve as a new target for trastuzumab-resistant HER2+ breast cancer treatment.

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