Deciphering the role of ErbB2/HER2 in cancer cell lines: a proto-oncogene with antiapoptotic activity

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HER2 (ErbB2/NEU) is a transmembrane tyrosine kinase belonging to the ERBB family of receptors. HER2/ErbB2 is also a well known proto-oncogene which is one of the most practiced molecules in the cancer area. A primary function of HER2 is suppressing apoptosis to enhance cell survival giving rise to uncontrolled proliferation and tumor growth. Caspases are well renowned proteases which are initiators as well as perfect finishers of the apoptotic process. The primary objective of this research was to study the expression levels of HER2 and other factors related to apoptosis like CASP-3 and CASP-8 in several cancers including breast and other cancer cell lines and eventually to find a significant correlation between all these. Finally we obtained a result which clearly showed an increase in expression of HER2 in all cancer cell lines as compared to that of CASP-3 and CASP-8. As an addition to our present study, we also analyzed different natural miRNAs acting on ErbB2 and CASP3 using different miRNA databases. The target elements for miR-548d-3p and miR-559 were revealed by studying the bioinformatical analysis of the 3’-UTRs of ErbB2. It was also found that hsa-miR-375 is a common miRNA that binds to 3’-UTR region of both ErbB2 as well as to CASP-3 and in future we are in a plan to explore the role of these miRNAs in relation to ErbB2 and caspases. In summary we conclude that HER2 promotes cell survival by inhibiting apoptosis i.e. by downregulating CASP-3 and CASP-8. This research is a novel study and is expected to aid in better establishment of correlation between HER2 and caspases in different cancers.

Keywords: HER2/ErbB2; caspase-3; caspase-8; apoptosis; cancer cell line


Introduction

ErbB2 (HER-2/NEU) is known to encode a transmembrane glycoprotein and shows an extensive homology to the human epidermal growth factor receptor i.e. ERBB2 protein [1]. At the same time, it is a well renowned oncprotein belonging to the EGFR family playing an important role in proliferation, cell growth and differentiation [2, 3]. ErbB2 receptor regulates several important functions which are controlled by any of the ErbB-receptor family members like cell growth, differentiation, and apoptosis [4]. The ErbB2 gene also has an essential role in human malignancies. The overexpression of HER2 has been found to be related to the aggressiveness of the disease, increased mortality and higher relapse ratio [5-8]. ERBB tyrosine receptor kinases activate several pathways, including PI3K-AKT and RAS-MAPK pathways, which regulate many cell functions including proliferation, migration, survival, and cell growth among others [9]. Hyperactivation or overexpression of these receptors can lead to uncontrolled cell growth and proliferation leading to cancer development. At the same time, overexpression of HER2 initiates the induction of downstream signaling cascade(s), like phosphatidylinositol 3-kinase (PI3 K), leading to the
induction of Akt pathway, a known serine/threonine kinase which triggers the life expectancy of the cell [10, 11]. The role of HER2 in human tumors is really noteworthy. HER2, specifically, shows overexpression in 20-30% of breast cancers and it is likely to be associated with poor patient outcomes [7, 8, 12]. It is also overexpressed in many other types of human malignancies. The overexpression of HER2 also indicated increased metastatic characteristic of cancer cells [4]. One of the trademark features of cancer is the resistance to apoptosis [13, 14] and HER2 overexpression leads to suppression of apoptosis in breast cancer cells [15]. In order to suppress apoptosis, overexpression of HER2 has been shown to disrupt both the intrinsic and extrinsic apoptotic pathways through different mechanisms. It is also required to maintain HER2 expression for HER2-mediated suppression of apoptosis [9]. HER2 mediates activation of PI3K-AKT signaling which plays an important role in suppression of apoptosis by HER2. HER2 also negatively regulates p53 function. HER2 also suppresses p53 by different indirect mechanisms that are both mediated by AKT [16, 17]. The inhibition of p53 expression eventually inhibits apoptosis as p53 has been shown to upregulate several pro-apoptotic genes involved in the intrinsic apoptotic pathway [18-21]. Survivin seems to play an important role in HER2-mediated apoptosis suppression as it has a strong association with HER2 expression in patient tumors [9, 22]. It has been shown that knockdown of HER2 reduces survivin drastically [23] and vice-versa is also true i.e forced expression of HER2 increases survivin [24, 25]. All in all these results clearly indicate that HER2 expression promotes survivin expression that indirectly leads to decreased apoptosis. HER2 also targets Bcl-2 in promoting cell survival. There is an upregulation of Bcl-2, Bcl-xl, Mcl-1 and survivin due to overexpression of HER2 [25-27].

Apoptosis is a programmed mechanism by which the cells are allowed to commit suicide and is genetically oriented [28-31]. It is indispensable in development and homeostasis. Apoptosis helps in getting rid of damaged or infected cells which might interfere with normal functioning of cells [32, 33]. Several cysteine aspartate-specific proteases which are called as caspases cleave their respective substrates leading to cellular demise during this apoptotic process [34]. There are two major well-studied apoptotic processes i.e the extrinsic and intrinsic pathways [29, 35, 36]. The initiation of intrinsic pathway takes place by release of cytochrome c which is a component of the mitochondrial electron transport chain from the mitochondrial intermembrane space in response to cell stress. It then leads to the apoptosome structure construction, consisting of procaspase-9, cytochrome-c and Apaf-1 that gives rise to caspase-9 activation, then triggering caspase-3 and thus DNA fragmentation finally [37, 38]. The outer mitochondrial membrane loses its integrity in a process termed outer mitochondrial membrane permeabilization (MOMP) that causes release of second mitochondria-derived activator of caspase (SMAC or DIABLO). Furthermore these proteins appreciably increase the caspase activity by preventing caspase inhibition by inhibitor of apoptosis proteins (IAPs). XIAP likely plays a significant role as knockdown of XIAP in HER2-overexpressing cells enhanced apoptosis by TRAIL [39]. On the other hand, the extrinsic pathway is known to be mediated by TNFR, Fas and TRAIL which are sub-group of Tumor Necrosis Factor receptors (TNFR) superfamily. Furthermore, initiator caspases such as caspases 8 and 10 are initiated and recruited by the activation of these death receptors. Moreover, it also leads to the formation and activation of other complexes i.e death inducing signaling complex (DISC) which ultimately results in the activation of an effector caspase typically caspase 3 and apoptosis [40]. An upregulation of HER2/ErbB2 has also been reported in studies in node positive breast cancer patients [7, 8]. There is difference in expression of HER2 at different levels in breast cancer cell lines taken from different patients [41]. There are some studies which report an association of overexpression of the HER2 gene with an ER-negative characteristic and with decreased ER and PR densities in the cancer cell cytoplasm [42].

In our study nine different cell lines were included for their HER2/ErbB2, CASP3/8 expressions. We calculated Ct values of HER2, CASP3/8 of these cell lines as relative expression in Real-time PCR. According to relative expressions of HER2, CASP3/8 in cell lines calculated with 2-ΔΔCt method, it was observed that HER2 expression was higher as compared to CASP-3 and CASP-8 expression in all selected cell lines such as MCF7 (breast cancer), HCC1500 (breast cancer), CRL1500 (breast cancer), MDA-MB-231 (breast cancer), A549 (lung cancer), DU145 (prostate cancer), HepG2 (hepatocellular carcinoma), HGC-27 (gastric cancer) and HeLa (cervix cancer). It was also noticed that expression of CASP-3 was higher than CASP-8 in all the selected cell lines. Overexpression of HER2 has been noticed in several types of primary human tumors, which includes 25-30% of breast and has also been correlated with tumor growth and adverse prognosis [43, 44]. Thus HER2 overexpression is noticed to be as a factor in the pathogenesis of several cancers [45].

An increase in expression levels of HER2 in many breast cancer cell lines and its correlation with increase in resistance to apoptosis was shown by Yu et al. [41]. These findings are consistent with our results. ER-negative breast cancer cell line has also been stated to be associated with overexpression of the HER2 gene [43]. We also found higher expression of HER2 in MDA-MB-231 which is an ER(-) breast cancer cell line while there was decrease in expression of CASP3/8.
HER2 binds to Neuregulin-1 (a protein essential to cell cycle survival) and initiates a series of signals in order to maintain cell survival and prevent apoptosis [46]. This prevention of apoptosis is done by downregulating CASP3/8 which also correlates with our results. In a recent study it has been found that HER2/ErbB2 blocks apoptosis in breast cancer cells which is Taxol-induced by upregulation of p21Cip1, which participates in the negative regulation of Taxol-mediated p34Cdc2 activation required for Taxol-induced apoptosis [15]. This shows that HER2 suppresses the expression of CASP3/8 in different cancers which also holds true for the results that we found in different cancer cell lines. The MDA-MB-435 human breast cancer cell line contains only one copy of the ErbB2 gene per haploid and thus expresses low levels of ErbB2 [47]. In our experiment we selected MDA-MB-231 human breast cancer cell which also showed lesser expression of ErbB2 as compared to other selected breast cancer cell lines. Interestingly we also noticed a comparatively higher expression of CASP3/8 in MDA-MB-231 human breast cancer cell as compared to other breast cancer cell lines. It implies that the lower expression of HER2 in MDA-MB-231 breast cancer cell line has a difficult task in suppressing the expression of CASP3/8. In our research, caspase-3 was not expressed to an appreciable amount in MCF7 cells, which is consistent with results reported by Xiu-fang Wang et al. and Mathiasen et al. [48, 49]. The expression of HER2 in contrast to CASP3/8 is also elevated in lung cancer cell line, A549. Previous reports also suggested that amplification/overexpression of the HER2/neu gene to be associated with cancers of the lungs [50-52]. In a very recent study, a study was conducted on non-small cell lung cancer and was shown that HER2 downregulate expression of apoptosis inducing factor in this cancer type [53]. It also correlates with our study as there is low expression of CASP3/8 as compared to HER2 in A549. The role of HER2 to inhibit apoptosis in lung cancer was further confirmed by Ren et al. as they carried out an experiment involving HER2/neu siRNA-mediated gene silencing on cell cycle as well as apoptosis of lung adenocarcinoma cells [54, 55].

The prostate cancer cell line (DU145) also showed an increased over expression of HER2 than CASP3/8 indicating role of HER2 in prostate cancer development by inhibiting CASP3/8 activity. An increase in serum HER2/neu in prostate cancer was also stated by Siyanpanopolou et al. The release soluble extracellular domain (ECD) takes place in the serum of prostate cancer patients by the overexpression of HER2/neu [56]. Till date, there has been no relation found for CASP3/8 in prostate cancer cell lines. In this study, we are most probably the first to show down regulation of CASP3/8 in prostate cancer cell line (DU145). An increase in expression of HER2 and decrease in expression of CASP3/8 in hepatocellular carcinoma cell line i.e. HepG2 was also found. This particular finding complements with the experiment carried out by Zhang et al. [57] but contradicts with the findings of Potti et al. and Z-H Xian et al. [58, 59]. But none of them carried out their experiment using hepatocellular carcinoma cell line i.e. HepG2. As far our knowledge, we used hepatocellular carcinoma cell line HepG2 for the first time in literature in order to study expression studies of HER2 and CASP3/8.

Apart from the above discussed cancer cell lines, cancer of gastrointestinal tract has been also found to be associated with amplification or overexpression of the HER2 [60, 61]. An increase in HER2 and corresponding decrease in expression of CASP3/8 is also noticed in present study. The decrease in expression of CASP3/8 indicates that it has been suppressed by HER2 in HGC-27, a gastric cancer cell line. Recently it has been demonstrated that caspase-3 has an inverse effect on HER2 in human gastric cancer. Zhang et al. showed that HER2-targeted induction of apoptosis with anti-HER2 antibody and caspase-3 fusion protein triggered inhibition of human gastric cancer potentially and this totally complemented our results [62]. Recently gastric biopsy samples of patients with gastric carcinoma diagnosis showed the overexpression of HER2/neu [63]. Overexpression of HER2 has also been found in cervical cancer [64]. Increase in expression of HER2 and corresponding decrease in expression of CASP-3 and CASP-8 is found in our study too. We used HeLa cells as cervical cancer cell line. The reverse role or expression of HER2 and caspase-3 in HeLa cells has also been demonstrated by Shen et al. [65]. But some of the studies mention lower expression of HER2 in cervical cancer which contradicts our studies [66].

In addition to this, we tried to analyze different natural miRNAs acting on ErbB2 and CASP3. We made a thorough study of different literatures and different databases like DIANA Tools, miRTarBase, miRanda, Targetscan and STarMir. The target elements for miR-548d-3p and miR-559 were revealed by studying the bioinformatical analysis of the 3’-UTRs of ErbB2. Apart from this, it has been stated that both miR-548d-3p and miR-559 can interact specifically with the 3’-UTR of the ERBB2 mRNA. It was shown by a predicted miRNA/mRNA interaction experimental validation [11]. By studying TargetScan (http://www.targetscan.org), we analyzed certain miRNAs which showed 5mer seed match with ErbB2 like hsa-miR-4650-5p, hsa-miR-4516, hsa-miR-4434, hsa-miR-4455 while some miRNAs showed 7mer-m8 seed match with ErbB2 like hsa-miR-1207-5p, hsa-miR-4763-3p, hsa-miR-593, hsa-miR-3664-5p etc. By investigating miRNA databases, we found a miRNA i.e hsa-miR-375 which binds to 3’-UTR region of both ErbB2 as well as to CASP-3. However this miRNA is poorly
conserved for ErbB2 and binds to position 364-370 of ERBB2 3' UTR. miRanda also showed target sites of hsa-miR-375 to 3'-UTR region of CASP3. We will be targeting this particular miRNA in order to study ErbB2 and CASP3 in different cancer cell lines and tissues. We hope the result will be quite informative and boosting in the field of microenvironment related to cancer. In future, we will be trying to study the effect of these miRNAs on transfecting cancerous cell lines which show an overexpression of ErbB2. We will also interrelate these results to CASP-3 and CASP-8 as a mechanism to curtail ErbB2/Her2 overexpression in cancers. These data along with our established data in recent research might provide molecular basis for the better establishment of correlation between HER2 and caspasas in different malignancies and our future research plan targets on the application of miRNAs in ERBB2-targeted therapy.

Conclusions

Our recent study showed an upregulation of HER2/ErbB2 in breast cancer cell lines (MCF7, HCC1500, CRL1500, MDA-MB-231), lung cancer cell line (A549), prostate cancer cell line (DU145), hepatocellular carcinoma (HepG2), gastric cancer cell line (HGC-27) and cervix cancer cell line (HeLa). HER2/ErbB2 being a well-known protooncogene, tries to evade apoptosis via downregulating CASP3/8. The downregulation of CASP-3 and CASP-8 was noticed in all of the cancer cell lines being used. In fact overexpression of HER2/ErbB2 in cancer cell lines promotes tumor survival by curtailing the expression of CASP-3 and CASP-8. This gives an idea that HER2/ErbB2 has a significant role in tumorigenesis in many cancers due to its antiapoptotic role.

All the data discussed or pointed in this paper as well as supported by other reports reveals the fact that many human cancer cell lines upregulate HER2/ErbB2 expression that help to maintain its oncogenic property via inhibiting apoptotic activity of CASP3/8. Thus our findings further strengthen the knowledge of inverse correlation between HER2 and CASP3/8 in different cancer cell lines. In summary, the results in this study reveal a significant role of HER2 on the cell proliferation of human cancers through apoptosis inhibition by downregulating CASP3/8. To our knowledge, this is for the first time in literature that expression level of HER2 and caspase-3 have been studied in so many cancer cell lines simultaneously. We also included caspase-8 in our expression study which makes it more prominent and authentic. The expression level study of HER2 with inclusion of caspase-8 had never been studied earlier. All in all we expect that this study might pave the way for better understanding the antiapoptotic role of HER2/ErbB2 in many malignancies. Furthermore our data can also be utilized in order to make sound comment for the relationship between HER2 and CASP-3 and CASP-8 in different cancers.

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Conflict of interest

Authors have no conflict of interest regarding the subject of this manuscript.

References


