Cetuximab Enhances the Anti-proliferative Effect of Trastuzumab in ERBB2 Over-expressing Breast Cancer Cells – Preliminary Study

Cetuximab zvyšuje antiproliferativní účinek trastuzumabu u buněk karcinomu prsu s nadměrnou expresí ERBB2 – předběžná studie

Überall I.[1,2], Křížová K.[1,2], Steigerová J.[1,2]

[1] Institute of Clinical and Molecular Pathology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic
[2] Institute of Molecular and Translational Medicine (IMTM), Faculty of Medicine and Dentristry, Palacky University, Olomouc

Summary

Background: The tyrosine kinase receptor comprises a subclass of cell surface growth factor receptors. Inhibition of certain members of the Epidermal Growth Factor Receptor (EGFR) family is an effective treatment approach in some cancers. The anti-tumor effects are greater when this approach is combined with inhibition of the ERBB2 receptors. These studies provide novel experimental data demonstrating a significant augmentation of the anti-proliferative effects of monoclonal antibodies (cetuximab and trastuzumab) on human breast carcinoma cell lines with different level of ERBB receptor expression. ***Materials and Methods:*** Three breast cancer cell lines, MCF-7, BT-474, and SK-BR-3 were used. These are characterised by different levels of EGFR and/or other ERBB family members. Inhibition of cell growth in response to cetuximab, trastuzumab or their combination was assessed by MTT assay. ***Results:*** The breast cancer cell lines differed in their sensitivity to TZ, CTX and their combination. The SK-BR-3 cancer cell line was sensitive to TZ. On the other hand, CTX had no effect on BT-474 or on SK-BR-3 that expressed low levels of EGFR and high levels of ERBB2. ***Conclusion:*** Our new experimental data show that the combination of anti-EGF receptor and anti-ERBB2 mAb may inhibit cancer cells expressing both EGF and ERBB2 receptors.

Key words

ERBB receptors – dual inhibition – trastuzumab – cetuximab – breast cancer

Souhrn

Východiska: Receptorové tyrozinkinázy představují podtřídu transmembránových receptorů. Inhibice členů rodiny receptorů pro epidermální růstové faktory je efektivní pro léčbu některých typů nádorů. V kombinaci s inhibicí ERBB2 receptorů je protinádorový efekt výraznější. Tato studie předkládá nová experimentální data, která prokazují zesílení anti-proliferačního účinku monoklonálních protilátek (cetuximab a trastuzumab) na nádorové linie odvozené z karcinomu prsu, které se liší rozdílnou expresí ERBB receptorů. ***Materiál a metody:*** Tato studie se věnuje kombinovanému účinku souběžné inhibice dvou členů rodiny ERBB, receptorů EGFR a ERBB2 pomocí monoklonálních protilátek trastuzumab (Herceptin, TZ) a cetuximab (Erbitux/C225, CTX). V rámci studie byly testovány tři buněčné linie odvozené z karcinomu prsu (MCF-7, BT-474 a SK-BR-3). Tyto linie se liší různou expresí EGFR a ERB2. Inhibice buněčného růstu byla sledována využitím metody MTT assay. Rovněž byly pomocí Western blotu analyzovány změny v expresi EGFR, ERBB2 a klíčových regulátorů buněčného cyklu. ***Výsledky:*** Prsní nádorové linie vykazovaly rozdílnou citlivost k působení TZ a CTX a jejich kombinací. Linie SK-BR-3 byla citlivá k působení TZ. Naopak CTX neměl vliv na linii BT-474 a na SK-BR-3, které exprimují nízkou hladinu EGFR a vysokou hladinu ERBB2. ***Závěr:*** Získané výsledky potvrzují hypotézu o vzájemném zesilujícím anti-proliferačním efektu současné inhibice obou receptorů.

Klíčová slova

ERBB receptory – duální inhibice – trastuzumab – cetuximab – karcinom prsu

This publication was supported by a grant of the Czech Ministry of Education MSM 6198959216, grant of Palacky University LF_2011_009 and the Biomedicine for regional development and human resources project (BIOMEDREG) C.1.05/2.1.00/01.0030.

Tato práce byla podpořena grantem MŠMT ČR MSM 6198959216, grantem Univerzity Palackého LF_2011_009 a Biomedicína pro regionální rozvoj a lidské zdroje (BIOMEDREG) C.1.05/2.1.00/01.0030.

Autoři deklarují, že v souvislosti s předmětem studie nemají žádné komerční zájmy.

The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.

Redakční rada potvrzuje, že rukopis práce splnil ICMJE kritéria pro publikace zasílané do biomedicínských časopisů.

The Editorial Board declares that the manuscript met the ICMJE "uniform requirements" for biomedical papers.

Ing. Bc. Ivo Überall, Ph.D.
Institute of Clinical and Molecular Pathology
Faculty of Medicine and Dentistry
Palacky University
Hněvotínská 3
775 15 Olomouc
e-mail: I.Uberall@seznam.cz

Obdrženo/Submitted: 8. 9. 2010
Přijato/Accepted: 7. 12. 2010

Introduction

The family of ERBB receptors includes the epidermal growth factor receptors, ERBB-1/EGFR/HER1, ERBB2/HER2, ERBB-3/HER3, and ERBB-4/HER4. These are transmembrane receptors with tyrosine kinase activity and they are frequently implicated in the development and progression of epithelial cell neoplasias both in animals and humans [1–3]. EGFR and ERBB2 play the key role. [4]. ERBB receptor activation occurs via ligand binding and dimerization initiated by molecular signals that promote tumorigenesis [5]. Clinical studies show that over-expression of EGFR and/or ERBB2, common in human cancers, correlates with poor prognosis [6].

Trastuzumab (Herceptin) and cetuximab (Erbitux/C225) are currently being investigated in clinical trials for their anti-tumor activity. These anti-EGFR and ERBB2 monoclonal antibodies have been approved by the FDA for treating tumors that express high levels of EGFR and ERBB2. Although some patients benefit from Erbitux and Herceptin treatment, failure may result, among other reasons, from a different expression/changed function of the multiple ERBB receptor family members leading to disharmony in receptor interaction. Cancers that co-express EGFR and ERBB2 have a poorer outcome than those that over-express either of the receptors alone. There is increasing evidence that pathways mediated by these receptors are being bypassed. A number of *in vitro* and *in vivo* studies suggest that dual inhibition of these receptors would be fruitful [7–11].

Ye et al (1999a) described the additive anti-proliferative effects of cetuximab (CTX) and trastuzumab (TZ) in the treatment of ovarian cancer cells [7]. Brockhoff et al (2004) investigated the different impact monoclonal inhibitors Cetuximab, Trastuzumab and Pertuzumab had on breast cancer cell lines. Cetuximab did not enhance inhibitory effect of Trastuzumab or Pertuzumab, most probably due to the dominant over-expression of ERBB2 [13].

In this study we tested the effect of simultaneous blockade of EGFR and ERBB2 on cell proliferation, cell survival and signal transduction in breast cancer cell lines, expressing various levels of EGFR and ERBB2 receptors.

Materials and Methods

Cell Lines

Three breast cancer cell lines, MCF-7, BT-474, and SK-BR-3 obtained from the American Type Culture Collection (Rockville, MD) were used. These are characterized by different levels of EGFR and/or other ERBB family members. BT-474 and SK-BR-3 breast cancer cell lines exhibit ERBB2 gene amplification and over-expression of EGFR is 3-fold higher in SK-BR-3 than BT-474 [13]. MCF-7 cell line had normal EGFR and ERBB2 expression and was used as a control [14]. They were maintained in DMEM medium supplemented with 10% fetal bovine serum, 100 units/mL streptomycin-penicillin, and incubated at 37 °C in an atmosphere of 95% air and 5% CO_2. Trastuzumab and cetuximab were applied in concentrations of 0.2, 2, 20 and 200 μg/mL. (IC_{50}, was achieved at a concentration (200 μg/mL TZ and 200 μg/mL CTX).

Growth Inhibition Assay

Inhibition of cell growth in response to cetuximab, trastuzumab or their combination was assessed by 2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as previously described [15]. Briefly, aliquots of cells (2×10^4 cells/mL) in DMEM/10% fetal bovine serum were plated in a 96-well culture plate with four replicates per treatment. After 24 hours of plating, cells were incubated at 37°C in the absence (control) or presence of cetuximab and/or trastuzumab for 72 hours as stated in the legends to figures. All incubations were terminated by addition of 10 μl of 0.5 g/mL stock 2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to each well. The reaction was allowed to proceed for 4 hours at 37 °C. The formazan crystals were dissolved by adding 100 μl 10% SDS, and the intensity of color was measured at 570 nm.

Western Blot Analysis

Cancer cell lines were plated in DMEM/10% fetal bovine serum in Petri dishes. After 24 hours of incubation, they were maintained for additional 72 hours in the absence (control) or presence of cetuximab (20 μg/mL) or trastuzumab (20 μg/mL). Treatment was terminated by adding a lysis buffer NP40 with protease and phosphatase inhibitors. The lysate was incubated for 30 minutes at 4 °C and then centrifuged at 15,000 g for 30 minutes at 4 °C. The supernatant was used for Western blot analysis after determination of protein concentration by the Bradford method [16]. Aliquots containing 50 μg of protein were separated on 10% SDS-PAGE and then electroblotted to a nitrocelulose membrane. The membrane was blocked 2 hours with 5% skimmed dried milk in TBS-T buffer (20 mmol/L TRIS (pH 7.5), 100 mmol/L NaCl, 0.1% Tween 20, followed by overnight incubation with the primary antibodies in TBS-T buffer containing 5% skimmed dried milk at 4°C (anti-mouse mcm7-1 : 2,000 Santa Cruz, anti-mouse EGFR-1 : 250 Neomarkers, anti-mouse ERBB2-1 : 250 Dako, anti-mouse-p27-1 : 250 Dako, anti-mouse cyclinA-1 : 250 Novocastra, anti-mouse cyclin B1 1 : 250 Novocastra, anti-mouse-cyclin-D1-1 : 250 Cell Signaling, anti-mouse Bcl-2 1 : 500, Biogenes). After washing three times with TBS-T buffer, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 2 hours at room temperature. Proteins were visualized using an enzyme-linked enhanced chemiluminiscence detection system (ECL, Amersham, Arlington Heights, IL). The membranes were then re-probed with mcm-7 antibodies as an internal control. Signals on the blots were visualized by autoradiography.

Results

Fig. 1 shows the concentration-dependent growth inhibition measured in % of viable cells, of breast carcinoma cell lines MCF-7, SK-BR-3 and BT-474 treated with an anti-EGFR (cetuximab) and/or anti-ERBB2 (trastuzumab) monoclonal antibodies.

MCF-7:TZ caused a slight increase in viable cells at 0.2 μg/mL, it caused no inhibition at 2, 20 or 200 μg/mL. CTX caused mild increase at 0.2 μg/mL but no increase at the dose of 2 μg/mL or 20 μg/mL

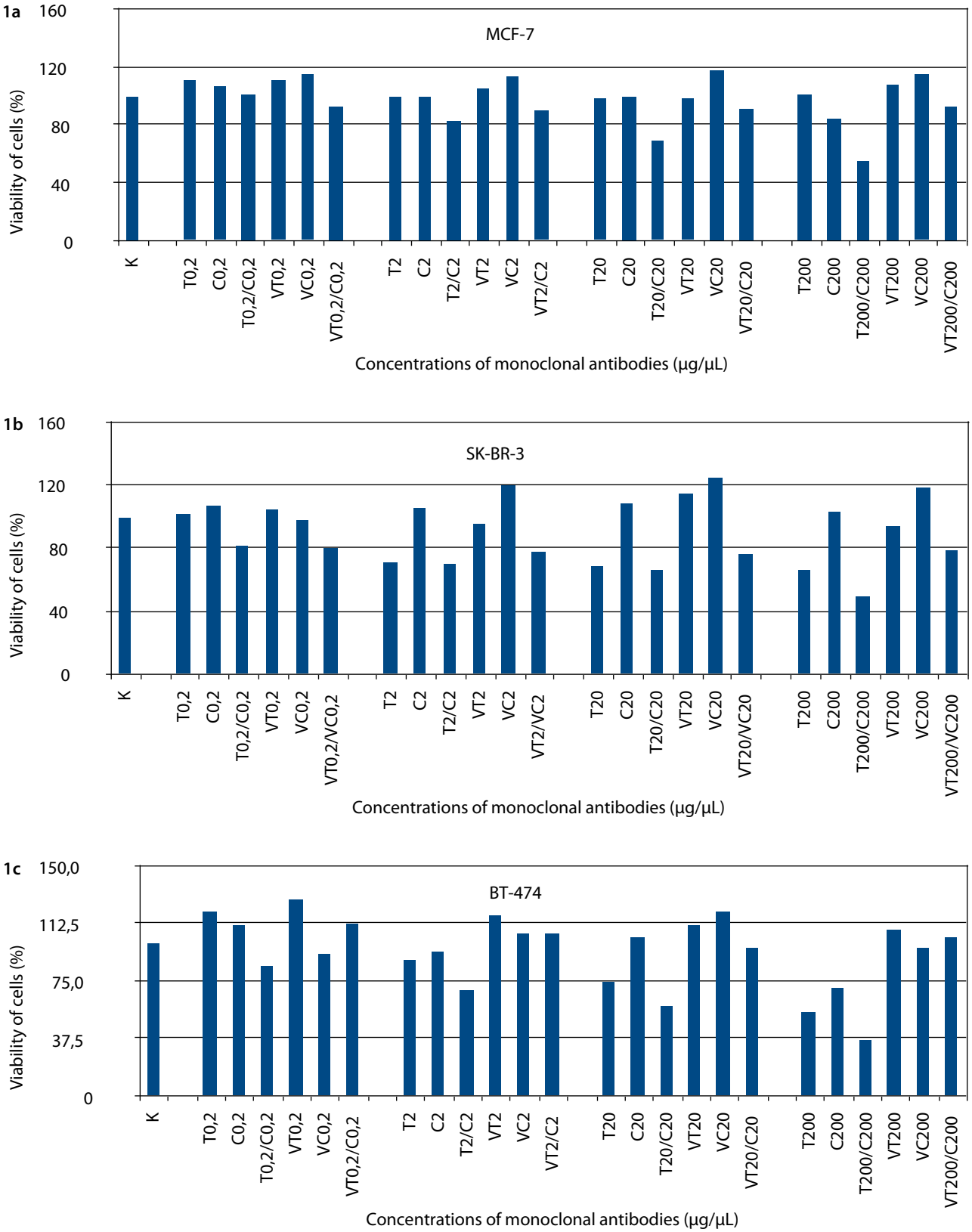

Fig. 1. Effect of monoclonal antibodies again EGF and ERBB2 receptors on cell viability in three human breast cell lines. The following concentrations of cetuximab (2-200 µg/mL) and trastuzumab (2-200 µg/mL) were applied to MCF-7 (Fig. 1a), SK-BR-3 (Fig. 1b), and BT-474 (Fig. 1c) cell lines for 72 h. VT = vehicle for different of trastuzumab concentrations, VC = vehicle for different of cetuximab concentrations). Results are expressed as the mean ± s. e. of three independent experiments.

and around 18% inhibition at the highest dose. The combined TZ + CTX shows linear decline in viable cells at 2 μg/mL, 20 μg/mL and a maximum at 200 μg/mL. SK-BR-3. There was 20% inhibition for TZ+CTX at 0.2 μg/mL, around 30% inhibition occurred at 2 μg/mL, 20 μg/mL and 200 μg/mL of TZ, there was no inhibition at any dose with C alone. However, TZ + C combination at the highest dose produced marked inhibition. BT-474: TZ rose to 120% at 0.2 μg/mL and then decreased below control values for the next three doses showing inhibition, CTX showed inhibition at the highest dose, inhibition produced by the TZ + CTX combination increased linearly from 2 μg/mL trough 20 μg/mL to 200 μg/mL. Overall, treatment of MCF-7, SK-BR-3, and BT-474 cell lines with combinations of CTX and TZ resulted in more significant growth inhibition than treatment with either antibody alone and this was concentration-dependent. 42% for MCF-7, 45% for SK-BR-3 and 62% for BT-474 control. The breast cancer cell lines varied in their sensitivity to TZ, CTX and their combination. The SK-BR-3 cancer cell line was sensitive to TZ. On the other hand, CTX had no effect on BT-474 or on SK-BR-3 that expressed low levels EGFR and high levels ERBB2.

A decrease in ERBB2 expression in MCF-7 line was observed after application of CTX and TZ alone and in combination. MCF-7 cell line did not express cyclin A. Exposure of SK-BR-3 to the combination resulted in more significant decrease in expression of cyclin A than single exposure. A similar relation was found in BT-474 cell line except that exposure of BT-474 cell line to CET resulted in complete loss of cyclin A expression. Decrease in cyclin D1 was observed in all three cell lines. BT-474 was the most sensitive with loss of expression after exposure to individual substances as well as their combination. The level of Bcl-2 proteins showed no change after 72 h exposure to mAb alone or in combination. Increase in p27 expression was observed after exposure of MCF-7 to CTX alone. In contrast to MCF-7, p27 expression was increased by exposure to CTX alone and in combination with TZ. Exposure of BT-474 to CTX and TZ resulted in decrease in p27.

Discussion

We examined the mechanisms by which EGFR and ERBB2 signaling regulates cell cycle progression in MCF-7, BT-474 and SK-BR-3 human breast carcinoma cells. ERBB2 is constitutively phosphorylated in BT-474 cells, suggesting that in these cells, the orphan receptor may be transactivated by ligand EGFR. We demonstrated that an interruption of the EGFR pathway by inhibition of a receptor results in disturbance of the cell cycle. This study was the first to demonstrate that growth is inhibited when human breast cancer cells co-expressing EGFR and ERBB2 are treated by a combination of CTX and TZ. These results suggest that simultaneous blockade of different regulatory pathways might result in more significant anti-tumor effect. The findings are in accordance with reports on combined TZ and CTX effects on ovarian carcinoma cells [7].

The complementary effect of TZ and CTX can be explained by action at a receptor level. Binding of TZ to subdomain IV of ERBB2 does not interfere with the dimerization loop involved in receptor association and thus does not interrupt cross-signalling activity [17]. The complementary effect might be facilitated by obstruction of ERBB2 interaction with ERBB3 or ERBB4 receptors, analogous to prostatic cell lines [18]. We found that susceptibility to trastuzumab targeted to ERBB2 directly depends on the level of co-expressed EGFR. This suggests that HER2 over-expression was the best single predictive marker, although combinations of two markers provided additional predictive information. CTX failed to show any significant or additional inhibitory effect in SK-BR-3 and BT-474 cells; this is most probably due to over-expression of ERBB2 in these cell lines. Cell proliferation analysis of MCF-7, BT-474, and SK-BR-3 breast cancer cell lines revealed that both TZ and CTX inhibit cell cycle and drive cells into quiescence and that TZ is more effective than CTX. Over-expression of ERBB2 increases the turnover of p27. Lenfering et al (2001) examined the effect of forced expression on the half-life of p27 in MCF-7 cells that had a single copy of the ERBB2 gene [19]. To study ERBB2 driven cell cycle progression, we used BT-474 and SK-BR-3 breast cancer cells which exhibit ERBB2 gene amplification [20] and are ERBB2 dependent.

Ye et al reported that a combination of trastuzumab and CTX had synergistic antiproliferative effects on ovarian cancers cells [7]. However, in these cells, EGFR con-

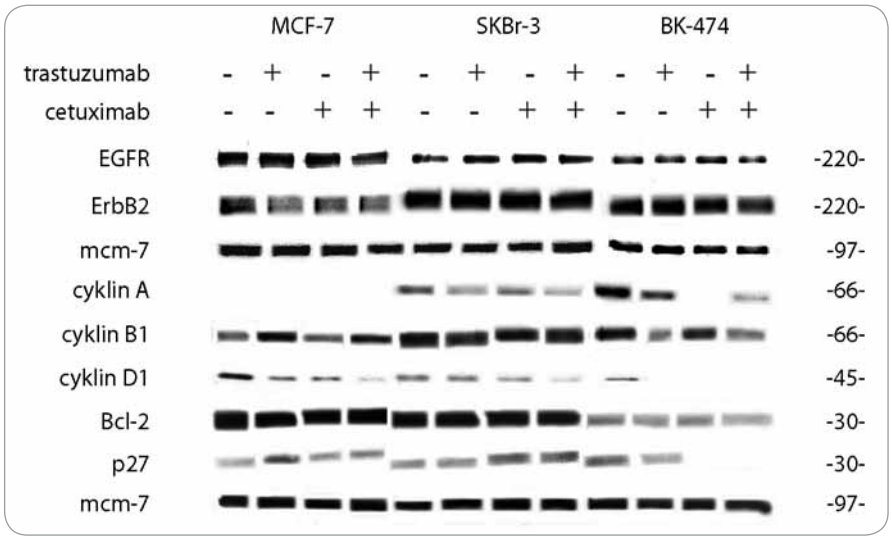

Fig 2. Western blot analysis of EGFR, ERBB2, cyclin A, cyclin B1, Bcl-2 and p27 in MCF-7, SK-BR-3, and BT-474 cells treated by cetuximab and trastuzumab. Cells were incubated in medium with cetuximab or/and trastuzumab for 72 h. The protein expressions of treated cells were compared with the protein expression of control, untreated cells. The expression of mcm-7 was used as a loading marker.

tent was higher than ERBB2 expression shown in other studies. Therefore, co-expression ratio of EGFR/ERBB2 would be conversely compared to them in breast cancer cell lines in this report. EGFR and ERBB2 both constitute biological targets for innovative treatment in breast cancer. Thus testing a combination therapy targeting these two receptors is of potential interest in prostate cancer. We are unable to anticipate the effects of a combination of the two drugs acting specifically on EGFR and ERBB2 from the results obtained with a single drug. We first addressed this question by an in vitro study using MCF-7, SK-BR-3, and BT-474 cancer cell lines. The results demonstrated that the CTX-TZ combination led to more intensive cytostatic/cytotoxic effects. The relative abundance of EGFR and ERBB2 may play a role in the final effect of dual receptor targeting since EGFR-ERBB2 heterodimers are a functionally potent signalling combination. ERBB2 over-expression reduced the EGFR internalisation rate thus increasing the fraction of EGFR recycled to the cell surface.

Nahta et al (2004) explored combined effects of TZ and pertuzumab (Omnitarg, 2C4) in a ERBB2 over-expressing BT-474 breast cancer cell line. BT-474 is a cell line with endogenous TZ-increased 2C4-mediated disruption of ERBB2 dimerization with EGFR and HER3 and both agents synergistically inhibited the survival of BT-474 cells, in part because of increased apoptosis [21]. Lenferink et al (2001) reported that over-expression of ERBB2 receptors reversibly reduced p27 and increased cyclin D1 levels. Therefore, ERBB2 blockade resulted in stabilization of p27, reduction in cyclin D1 and cell cycle arrest [19]. Several reports link signalling pathways activated by ERBB2 with regulators of cell cycle progression. Activation of Ras/MAPK results in degradation of p27 [22,23].

Molecular factors of cell proliferative and apoptotic pathways were examined in relation to cell survival analyses. The changes observed in these cellular factors mirror the findings for cell survival. It was shown that paradoxical reduction in negative regulators of cell division $p27^{Kip}$ was less marked with a combination of the drugs than with TZ and CTX alone. Differences in $p27^{Kip}$ expression in MCF-7 cell lines were not significant. TZ increased the half-life of $p27^{Kip}$ by decreasing cyclin E/cyclin-dependent kinase (CDK) 2 – mediated phosphorylation of $p27^{Kip}$ and blocking subsequent ubiquitin-dependent degradation. TZ also mediated an association between $p27^{Kip}$ and CDC2 complexes, resulting in G1 cell cycle arrest. Importantly, antisense oligonucleotides and siRNA that reduced $p27^{Kip}$ expression levels also blocked trastuzumab – mediated growth arrest of ERBB2 over-expressing SK-BR-3 breast cancer cells. Cellular localization of $p27^{Kip}$ might also be important for TZ response as shown in TZ-resistant BT-474 cell lines. ERBB2 overexpressing cells demonstrated loss of nuclear $p27^{Kip}$ expression. Thus, $p27^{Kip}$ could serve as a marker of TZ response and as a therapeutic target in a subset of breast cancers that show resistance to trastuzumab [24].

In summary, these studies provide novel experimental data demonstrating significant augmentation of anti-proliferative effects of TZ and CTX on human breast carcinoma cell lines MCF-7, SK-BR-3, and BT-474. Binding of mAb to both receptors may prevent formation of active receptor heterodimers. The results of this study provide exprimental evidence that the combination of anti-EGF receptor and anti-ERBB2 mAb may be useful in inhibiting cancer cells expressing both EGF and ERBB2 receptors.

References

1. Lohrisch C, Piccart M. An overview of HER-2. Semin Oncol 2001; 28 (6 Suppl 18): 3–11.
2. Berkovcová J, Hajdúch M, Dziechcierková M et al. Predikce účinnosti tyrozikinázových inhibitorů EGFR1 v léčbě nemalobuněčných plicních karcinomů. Klin Oncol 2006; 19(3): 171–176.
3. Bouchalova K, Cizkova M, Cwiertka K et al. Triple negative breast cencer – current status and prospective tergeted treatment based on HER1 (EGFR), TOP2 and C-MYC gene assessment. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2009; 153(1): 13–17.
4. Laskin JJ, Sandler AB. Epidermal growth factor receptor: a promising target in solid tumours. Cancer Treat Rev 2004; 30(1): 1–17.
5. Mass RD, Press MF, Anderson S et al. Evaluation of clinical outcomes according to HER-2 detection by fluorescence in situ hybridization in women with metastatic breast cancer treated with trastuzumab. Clin Breast Cancer 2005; 6(3): 240–246.
6. Slamon DJ, Clark GM, Wong SG et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235(4785): 177–182.
7. Ye D, Mendelsohn J, Fan Z. Augmentation of a humanized anti-HER2 mAb 4D5 induced growth inhibition by a human-mouse chimeric anti-EGF receptor mAb C225. Oncogene 1999; 18(3): 731–738.
8. Moulder SL, Yakes TM, Muthuswamy SK et al. Epidermal growth facor receptor (HER1) tyrosine kinase inhibitor ZD1839 (Iressa) inhibits HER/neu (ERBB2)-overexpressing breast cancer cells in vitro and in vivo. Cancer Res 2001; 61(24): 8887–8895.
9. Moasser MM, Basso A, Averbuch SD et al. The tyrosine kinase inhibitor ZD1839 („Iressa") inhibits HER2-driven signaling and supresses the growth of HER2-overexpressing tumor cells. Cancer Res 2001; 61(19): 7184–7188.
10. Normanno N, Campiglio M, De LA et al. Cooperative inhibitory effect of ZD1839 (Iressa) in combination with trastuzumab (Herceptin) on human breast cancer cell growth. Ann Oncol 2002; 13(1): 65–72.
11. Ye D, Mendelsohn J, Fan Z. Androgen and epidermal growth factor down-regulate cyclin-dependent kinase inhibitor p27Kp1 and costimulate proliferation of MDA PCa 2a and MDA PCa 2b prostate cancer cells. Clin Cancer Res 1999; 5(8): 2171–2177.
12. Reid A, Vidal L, Shaw H, de Bono J. Dual inhibition of ErbB1 (EGFR/HER1) and ErbB2 (HER2/neu). Eur J Cancer 2007; 43(3): 481–489.
13. Brockhoff G, Heckel B, Schmidt-Bruecken E et al. Differential impact of Cetuximab, Pertuzumab and Trastuzumab on BT-474 and SK-BR-3 breast cancer cell proliferation. Cell Prolif 2007; 40(4): 488–507.
14. Cuello M, Ettenberg SA, Clark AS et al. Down-regulation of the ERBB2 receptor by trastuzumab (herceptin) enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in breast and ovarian cancer cell lines that overexpress ERBB2. Cancer Res 2001; 61(12): 4892–4900.
15. Romijn JC, Verkoelen CF, Schroeder FH. Application of the MTT assay to human prostate cancer cell lines in vitro: establishment of test conditions and assessment of hormone-stimulated growth and drug-induced cytostatic and cytotoxic effects. Prostate 1998; 12(1): 99–110.
16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1967; 72: 248–254.
17. Badache A, Hynes NE. A new therapeutic antibody masks ERBB2 to its partners. Cancer Cell 2004; 5(4): 299–301.
18. Mellinghoff IK, Vivanco I, Kwon A et al. HER2/neu kinase-dependent modulation of androgen receptor function through effect on DNA binding and stability. Cancer Cell 2004; 6(5): 517–527.
19. Lenferink AE, Busse D, Flanagan M et al. ERBB2/neu kinase modulates cellular p27(Kip 1) and cyclin D1 through multiple signaling pathways. Cancer Res 2001; 61(17): 6583–6591.
20. Alimandi M, Romano A, Curia MC et al. Cooperative signaling of ErbB3 and ErbB2 in neoplastic transformation and human mammary carcinomas. Oncogene 1995; 10(9): 1813–1821.
21. Nahta R, Hung MC, Esteva FJ. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. Cancer Res 2004; 64(7): 2343–2346.
22. Kawada M, Yamagoe S, Murakami Y et al. Induction of p27Kip1 degradation and anchorage independence by Ras through the MAP kinase signaling pathway. Oncogene 1997; 15(6): 629–637.
23. Rivard N, Boucher MJ, Asselin C et al. MAP kinase cascade is reguired for p27 downragulation and S phase entry in fibroblastis and epithelial cells. Ann J Physiol 1999; 277(4 Pt 1): C652–C664.
24. Lane HA, Beuvink I, Motoyama AB et al. ErbB2 potentiates breast tumor proliferation through modulation of p27Kip1-Cdk2 complex formation: receptor overexpression does not determine growth dependency. Mol Cell Biol 2000; 20(9): 3210–3223.