The Effect of Tyrosine Kinase Inhibitors on Prostate Cancer Cell Growth

TİROZİN KİNAZ İNHİBİTÖRLERİNİN PROSTAT KANSER HÜCRESİNDE BÜYÜME ÜZERİNE ETKİSİ

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Summary

Purpose: Prostate cancer is characterised by eventual loss of hormonal sensitivity with increased aggressive behaviour of the tumour. As the tumor progress, tissue growth factors, such as epidermal growth factor, appear to be more effective than steroid hormones in the control of cancer cells. In this study, inhibitors of tyrosine kinases are investigated in terms of their effect on prostate cancer cell proliferation.

Materials and Methods: Two immortal human prostate cancer cell lines, PC3 and DU145, were routinely cultured at 37oC in atmospheric air enriched with 5% C02 with 10% foetal calf serum. Cells' proliferations were determined by MTT assay.

Result: The proliferation of PC3 cell line was increased by epidermal growth factor (EGF) administration ($p<0.05$) while DU145 cell line was insensitive. However, both cell growth were inhibited by the treatment of both natural and synthetic tyrosine kinase inhibitors in vitro ($p<0.001$).

Conclusion: In conclusion, EGF is an important autocrine growth modulator in prostate carcinoma therefore further studies which should target it's tyrosine kinase activity might be beneficial in the treatment of drug-resistant patients with prostate carcinoma.

Key Words: Prostate cancer, EGF, Tyrosine kinase

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Özet

Amaç: Prostat kanseri, hormonal sensitivitesindeki azalma ile birlikte, artmış agresif tümör karakteristiğine sahiptir. Tümör ilerledikçe epidermal büyüme faktörü gibi doku büyüme faktörleri, kanser hücrelerinin kontrolü üzerinde steroid hormonlardan daha etkili hale gelir. Bu çalışmada tirozin kinaz inhibitörlerinin prostat kanser hücresi büyümesi üzerine olan etkisi araştırılmıştır.

Materyal ve Metod: İmmortal prostat kanser hücreleri, PC3 ve DU145, standart hücre kültürü koşullarında %10'luk serum ile üretildi. Hücre proliferasyonu ise MTT proliferasyon metodu ile belirlendi.

Bulgular: PC3 prostat kanser hücrelerinin proliferasyonu epidermal büyüme faktörü ile uyarılırken ($p<0.05$), DU145 hücreleri bu faktöre karşı duyarsız bulundu. Bununla birlikte her iki prostat kanser hücre tipinin çoğalması hem doğal tirozin kinaz inhibitoria, hemde sentetik tirozin kinaz inhibitörleri tarafından önlendi ($p<0.001$).

Sonuç: Sonuç olarak, epidermal büyüme faktörünün prostat kanserinde önemli bir otokrin büyüme faktörü olduğu ve onun tirozin kinaz aktivitesini hedefleyecek ileriki çalışmaların, tedaviye dirençli prostat kanseri vakalarmın tedavisinde faydalı olabileceği düşünüldü.

Anahtar **Kelimeler:** Prostat kanseri, EGF, Tirozin kinaz

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The incidence of prostate cancer (PC) has been dramatically increased in past decades and is becoming the most commonly diagnosed cancer in men in the Western world (1). The highest mortality rates from PC occur in the black male population in USA, nearly 120 times more than men in China and 30 times more than men in Japan. However, mortality rate is only two times higher than that of Japanese immigrants living in USA (2) suggesting that the differences are due more to enviromental than genetic factors. On the other hand, microscopic (latent) PC is commonly found, with similar frequency in these countries (3). Studies on the relationship between diet and risk for prostate cancer appear of special interest, since nutrition may well influence the hormone balance, especially of

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steroids (androgens) which have been implicated in the growth of PC.

It has been shown that polypeptide growth factors play an important role on the growth management of normal prostate (4), benign prostate hyperplasia (BPH) (5) and PC (6). Epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGRF) and insulin like growth factor receptor (IGFR), possess intrinsic tyrosine kinase activity (7). Overexpression of tyrosine (TK) activity has been detected in many carcinomas such as osteosarcoma (8), breast carcinoma (9), ovary carcinoma (10), bladder carcinoma (11) and PC (12). Increased productions of Epidermal Growth Factor (EGF) and overexpression of the EGFR have been associated with neoplastic transformation (13). Increased cellular proliferation rate has been shown in primary prostate cancer (five-fold) and in metastatic prostate cancer (twenty-four fold) when compared with normal prostate.

Considerable progress has been made in understanding the role of tyrosine kinase (TK) in the uncontrolled growth of neoplastic cells in culture. Therefore tyrosine kinases and their signalling pathways have been identified as a potential target for drug design. Several kind of inhibitors have been determined to inhibit the cellular TK activity, including the bioflavonoids, such as quercetin, lavendustin A, herbimycin A and genistein (7). Genistein (5,7,4'-thihydroxyisoflavone), an isoflavonoid found in soybeans, has been shown to inhibit the proliferative growth of human breast carcinoma, PC, epidermoid carcinoma and leukemia cell lines in vitro (14). Akiyama and associates (15) demonstrated that genistein is a highly specific inhibitor of TKs but rarely inhibits the activity of serine-theronine kinases and other ATP analogue-related enzymes. Several type of synthetic TK inhibitors were developed, such as tyrophostins, 2-thioindoles and quinazolines.

In this study, the growth regulation of PC cells by EGF in the presence of various TK inhibitors was investigated.

Material-Methods

Cell Culture

Two immortal human prostate cancer cell lines, PC3 and DU145, were used in this study. The cells were grown in RPMI-1640 medium containing 5 ug/ml phenol red, 25 mM HEPES and supplemented with 10% (v/v) foetal calf serum, 100 IU/ml Penicilin, 100 ug/ml Streptomycin. The cells were routinely cultured at 37°C in atmospheric air enriched with 5% $C0_2$. Genistein and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazofium bromide) was obtained from Sigma, Tyrophostin was obtained from Calbiochem. All tissue culture materials were obtained from Gibco.

MTT Assay

In order to check the growth effect of EGF and tyrosine kinase inhibitors on the cell lines, a colorimetric assay (MTT) was employed. MTT is a yellow water soluble tetrazolium salt which is reduced by the hydrogenase enzymes in the living cells into an insoluble purple formazan product. The method was originally described by Tim Mosmann (16) and modified by Carmichael et al (17). In the TK inhibitors studies, the cells were firstly treated with the inhibitors and then EGF was added to the medium. All inhibitors and EGF were prepared in phenol red free RPMI. All proliferation assays were performed in triplicate for 72h. According to EGF stimulation results, 10 ng/ml EGF was used in the inhibitor studies. The doses of quiazoline type TK inhibitors were 1,5, 10, 15 and 20 uM. The doses for tyrophostin type TK inhibitor were 1,5, 10, 25, 50, 100 uM. Genistein doses were 5, 10, 20, 50, 100 uM.

Statistical Analysis

Statistical analysis was performed by using SPSS package program for an IBM computer and the differences between groups were analysed by Mann-Whitney U test.

Results

Treatment with higher doses of EGF (7.5 and 10 ng/ml) resulted in a gradual increase ($p<0.05$) in proliferation of the PC3 cell line at 72h in comparison to the control (Figure 1). However, the treatment of DU145 cell line with EGF did not show any difference in growth at 72h (Figure 2). Cell proliferation rates were detected under EGF stimulation in the presence of inhibitors for 72h.

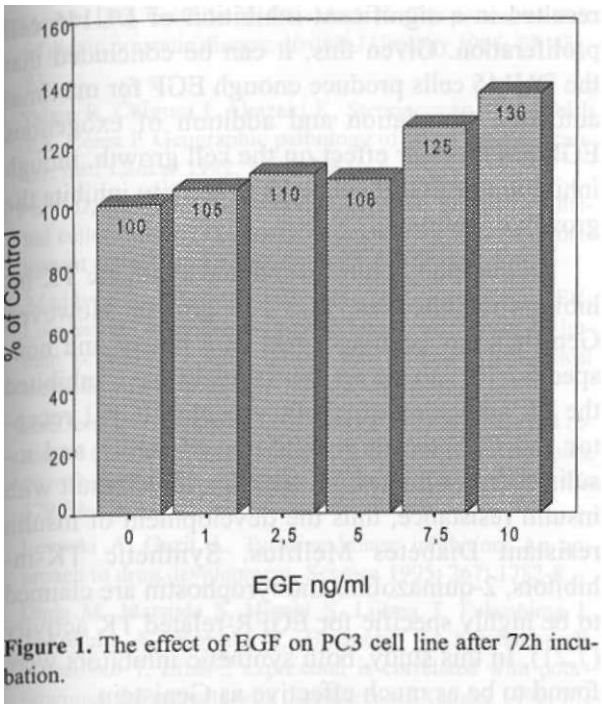

Figure 1. The effect of EGF on PC3 cell line after 72h incubation.

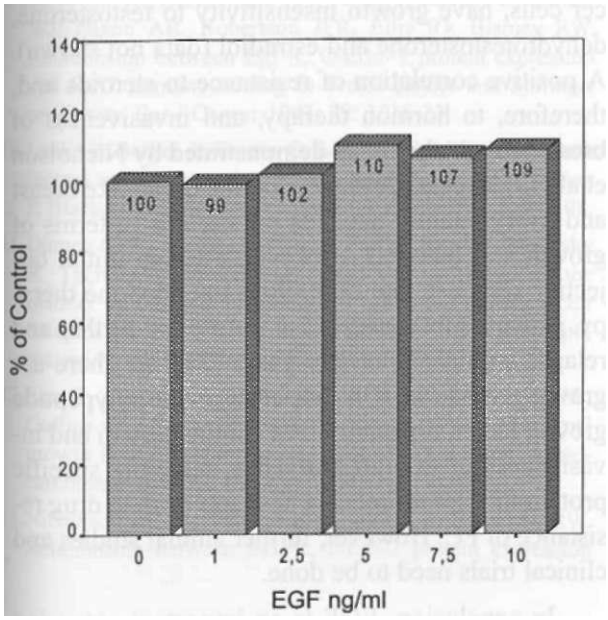

Figure **2.** The effect of EGF on DU145 cell line after 72h incubation.

Preliminary experiments showed that cell growth can be inhibited with micromolar concentrations and can be detected after both 48 and 72h. As a potent TK inhibitor, Genistein inhibited the growth of both cell lines. A 40% inhibition was revealed at 20 uM concentration in PC3 cell line (pO.OOl) (Figure 3). Higher doses of Genistein resulted with higher inhibition. DU145 cell growth was also inhibited by Genistein in a dose dependent manner ($p<0.001$, Figure 3).

Quanizoline type of EGFR-specific TK inhibitor led to a significant decrease of the proliferation of PC3 cells (pO.OOl). This TK inhibitor inhibited the growth of PC3 by 8.1% by 1 uM, 33.1% by 5 uM, and 75% by 10 uM (Figure 4). The TK inhibitor also inhibited the growth of DU145 cell line in a dose dependent manner (pO.OOl). DU145 exhibited 4, 32 and 51% inhibition by 1, 5, and 10 uM concentrations of the inhibitor, respectively (Figure 4).

Tyrophostin type of TK inhibitor was also inhibited both cell proliferations in a dose dependent manner and a marked inhibition started at 10 uM level (pO.OOl, Figure 5).

Discussion

Epidemiological studies have suggested the importance of diet on development and progression of cancer. Tyrosine Kinase activity has been found to be increased in many carcinoma including PC. EGFR-related TK activity is one of the important TK source in PC because of the growth sensitivity and the finding of increased secretion of its ligands (6). Several studies have revealed that there is a positive correlation between the tumour invasiveness, poor prognosis and EGFR overexpression in breast carcinoma (9) and in squamous cell carcinoma (18).

In this study, exogenous EGF stimulated the growth of PC3 cells but not DU145 cells, at least in short term. An inverse correlation between EGFR number and growth responsiveness to EGF has been demonstrated in breast cancer (19). Our laboratory results show that DU145 cell line has more EGFR than PC3 (unpublished results). This could be one of the explanation of the growth insensitivity of DU145 to EGF. In addition to that, DU145 cells secrete more EGF than PC3 (20). The growth insensitivity of DU145 cells in response to exogenous EGF might be attributed to high secretion of EGF and high expression of the receptor. Such a suggestion is supported by our observation that inhibition of endogenous EGFR-related TK activity

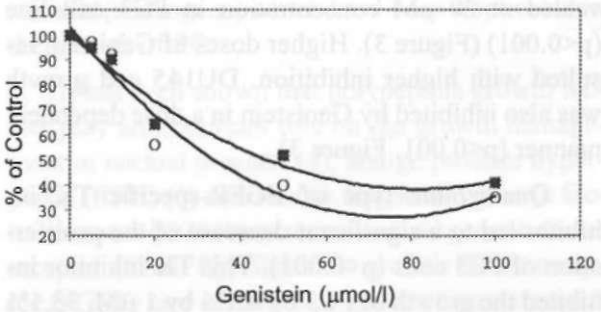

Figure 3. The effect of Genistein on PC3 (circle) and DU145 (square) cell lines after 72h incubation. The cells firstly treated with genistein and then EGF (10 ng/ml) added to the medium.

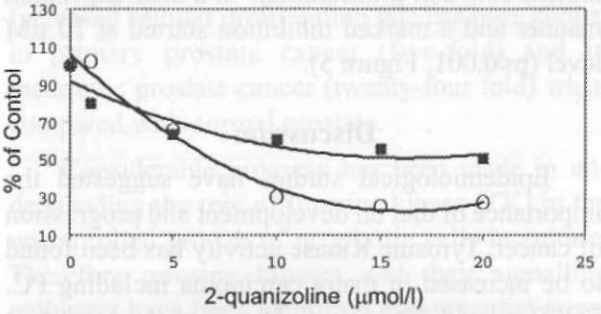

Figure 4. The effect of 2-quanisoline on PC3 (circle) and DU145 (square) cell lines after 72h incubation. The cells firstly treated with genistein and then EGF (10 ng/ml) added to the medium.

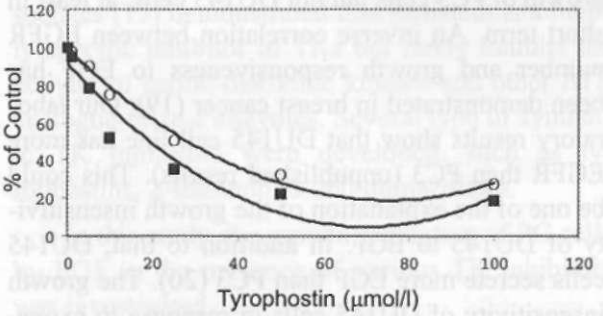

Figure 5. The effect of tyrophostin on PC3 (circle) and DU145 (square) cell lines after 72h incubation. The cells firstly treated with genistein and then EGF (10 ng/ml) added to the medium.

resulted in a significant inhibition of DU145 cell proliferation. Given this, it can be concluded that the DU145 cells produce enough EGF for maximal autocrine stimulation and addition of exogenous EGF has no clear effect on the cell growth, though inhibition of EGFR-related TK activity inhibits the growth of cell line.

In this study both natural and synthetic TK inhibitors inhibited the PC3 cell growth. However, Genistein has been accepted as a potent and non-specific TK inhibitor (15). It might have inhibited the TK activation of insulin receptor, IGF-1 receptor and FGF receptor. Inhibiton of IGF-1 and insulin receptor related TK activity could result with insulin resistance, thus the development of insulin resistant Diabetes Mellitus. Synthetic TK-inhibitors, 2-quinazoline and tyrophostin are claimed to be highly specific for EGFR-related TK activity (7,21). In this study, both synthetic inhibitors were found to be as much effective as Genistein.

Both cell lines, although they are prostate cancer cells, have growth insensitivity to testosterone, dehydrotestosterone and estradiol (data not shown). A positive correlation of resistance to steroids and, therefore, to hormon therapy, and invasiveness of breast tumours has been demonstrated by Nicholson et al (13). Prostate, as an endocrine organ like breast and ovary, mainly depends on steroids in terms of growth and function. However, after an initial objective response rate of 80-90% to endocrine therapy, patients subsequently fail to respond further and relapse within one or two years (22,23). There are growing evidences in implication of polypeptide growth factor, especially EGF, in the growth and invasiveness of PC (20,24). Thus, targeting specific protein-kinases promises a new approach to drug resistance of PC. However, further animal studies and clinical trials need to be done.

In conclusion, EGF is an important autocrine growth modulator in prostate carcinoma, therefore, further studies which should target it's tyrosine kinase activity might be beneficial in the treatment of drug-resistant patients with prostate carcinoma.

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