Lapatinib, a Dual Inhibitor of Epidermal Growth Factor Receptor (EGFR) and HER-2, Enhances Radiosensitivity in Mouse Bladder Tumor Line-2 (MBT-2) Cells In Vitro and In Vivo

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Background: The aim of this study was to evaluate the effect of lapatinib, a dual inhibitor of epidermal growth factor receptor (EGFR) and HER-2, on the radiosensitivity of murine bladder tumor line-2 (MBT-2) cells in vitro and in vivo.

Material/Methods: MBT-2 cells were pretreated with lapatinib at doses ranging from 200–1,000 nM for 30 min followed by radiation at doses ranging from 2.5–10 Gy for 30 min. A clonogenic assay (colony formation assay) assessed cell survival. Western blot measured phosphorylated epidermal growth factor receptor (p-EGFR), phosphorylated AKT (p-AKT), and phosphorylated HER-2 (p-HER2) and the apoptosis marker, PARP. The C3H/HeN mouse tumor xenograft model underwent subcutaneous injection of MBT-2 cells; mice were divided into four groups, treated with lapatinib (200 mg/kg), radiation (15 Gy), a combination of both, and with vehicle (control).

Results: Lapatinib pretreatment, combined with radiation, decreased MBT-2 cell survival, and suppressed radiation-activated levels of p-EGFR and p-HER-2. MBT-2 cells treated with a 10 Gy dose of radiation and 1000 nM of lapatinib showed combination index (CI) values of <1 indicating synergy. Increased expression of γ-H2AX, indicated increased apoptosis. In mice with tumor xenografts, a daily dose of lapatinib (200 mg/kg/day) for seven days combined with radiation on the fourth day suppressed tumor growth to a greater degree than radiation alone.

Conclusions: Lapatinib treatment enhanced the radiation sensitivity in an in vitro and in vivo murine bladder cancer model by decreasing radiation-mediated EGFR and HER-2 activation, and by causing DNA damage leading to cell apoptosis.

MeSH Keywords: Genes, erbB-1 • Genes, erbB-2 • Urinary Bladder Neoplasms

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/909865
Background

Worldwide, bladder cancer is one of the most common cancers of the urinary tract and is the fourth most common cause of cancer-associated deaths in elderly males in the USA [1,2]. Radiotherapy is an important first-line treatment for bladder cancer, particularly in cases where preservation of the bladder is required, and when patients are unsuitable for radical surgery [3]. At this time, the combination of radiation therapy or chemotherapy, with surgery, have shown limited improvement in patient survival outcomes for bladder cancer [4]. Cisplatin chemotherapy, combined with radiation therapy, has been shown to enhance the effects of radiation therapy in patients with bladder cancer [5]. However, the toxicities associated with chemotherapy make it a less favorable treatment option, especially in elderly patients and in patients with impaired renal function.

A proposed approach to improve the effects of radiotherapy is by selecting therapeutic agents that may suppress radiation activated signaling cascades [6]. A previously published study has shown that the epidermal growth factor receptor (EGFR) directed monoclonal antibody, cetuximab, in combination with radiotherapy, enhanced the overall survival (OS) rate and also improved local tumor control in patients with head and neck cancer [7]. The EGFR tyrosine kinase inhibitor, gefitinib showed a synergistic treatment effect when combined with radiotherapy in an in vivo study of bladder cancer [8]. However, the outcomes of combining both gefitinib and radiotherapy were not statistically significant and were limited only during the period during gefitinib treatment [8].

Previously published studies have shown that radiation-activated signaling molecules, including EGFR and human epidermal growth factor receptor 2 (HER-2) are over-expressed in bladder cancer and are associated with poor prognosis [9–11]. Therefore, it is possible that the role of EGFR and HER-2 in radiosensitization, and their inhibition may enhance the radiosensitivity of bladder cancer and may lead to the overall improved clinical outcome.

Lapatinib is a reversible and dual inhibitor of epidermal growth factor receptor (EGFR) and HER-2 and exerts its effects by inhibiting the binding of adenosine triphosphate (ATP) and inhibiting autophosphorylation to inhibit tumor cell proliferation and survival [12]. At this time, there have been no previous studies on the combination of irradiation with lapatinib in treating bladder cancer.

Therefore, the aim of this study was to evaluate the effect of lapatinib, a dual inhibitor of epidermal growth factor receptor (EGFR) and HER-2, on the radiosensitivity of murine mouse bladder tumor line-2 (MBT-2) cells in vitro and in vivo. The effects on MBT-2 cells of pretreatment with lapatinib were studied synergistically with irradiation, on apoptosis and DNA damage, as well as on tumor xenografts in a mouse model.

Material and Methods

Cell line and culture conditions

For this study, the mouse bladder tumor line-2 (MBT-2) was selected and obtained from the Radiation Oncology Department of Gastrointestinal and Urinary and Musculoskeletal Cancer, Liaoning Cancer Hospital and Institute, Shenyang, China. The cells were grown in RPMI-1640 culture medium supplemented with 50 U/ml penicillin/streptomycin and 10% fetal bovine serum (FBS) at 37°C in 5% CO₂ under humid conditions.

Reagents and chemicals

Lapatinib was obtained from GlaxoSmithKline Pharma (Zebulon, NC, USA). For the in vitro protocol, lapatinib (10 mM) was dissolved in 100% dimethyl sulfoxide (DMSO) and diluted further using culture media. For animal studies, lapatinib was mixed with Tween-80 (0.4%) and methylcellulose (0.5%) in water, and the animals received oral doses of lapatinib.

Irradiation of MBT-2 cells

Cells of the murine bladder tumor cell line, MBT-2, were exposed to radiation at doses between 2.5–10 Gy using a low energy 6 MV photon beam. Data were obtained at a distance of 100 cm from the source to the surface using an ionization chamber (cylindrical thimble).

Clonogenic assay (colony formation assay)

To test the effects of lapatinib and irradiation on colony formation, cells were seeded using six-well plates and a cell density of 1×10⁵ cells/well. The cells were exposed to different radiation doses, but received pretreatment with lapatinib (200–1,000 nM) for 30 min, with the control cells treated with dimethyl sulfoxide (DMSO). After pre-treatment with lapatinib, and following irradiation, the cells were cultured for a further week. Counting of the cell colonies was done using a light microscope (×100 magnification), and the colonies were defined as a group of 50 cells or more.

Western immunoblotting using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The cells were washed twice using ice-cold phosphate-buffered saline (PBS), followed by treating with lysis buffer (Sigma, USA). The separation of proteins from cell lysates was done...
by loading onto 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. The proteins were transferred to polyvinylidene difluoride membrane (PVDF) membranes. The blots were incubated with primary antibodies for 12 h at 4°C. The bound antibodies were analyzed with secondary antibodies. Enhanced chemiluminescence (ECL) (Thermo-Fisher, USA) was performed to identify the bands, according to the manufacturer’s protocol. The primary antibodies to EGFR, phosphorylated EGFR (p-EGFR), HER-2, and phosphorylated HER-2 (p-HER-2) were obtained from Abcam (Cambridge, MA, USA). The histone variant H2AX, phospho-H2AX, beta-actin, PARP and cleaved PARP were obtained from Cell Signaling, USA.

Cell cycle analysis

The cell cycle distribution was done by flow cytometry analysis. Propidium iodide (PI) staining for DNA in cells was analyzed. For the protocol, 10^6 cells/ml were exposed to lapatinib and irradiation as previously described and were collected after centrifugation. The cells were stained with PI (15 μg/ml) in PBS with 5 μg/ml DNase-free RNase and Tween-20 (0.5%). The samples were analyzed using an Attune™ NxT Acoustic Focusing Cytometer (Thermo Fisher, USA).

Immunofluorescence microscopic studies

The MBT-2 cells were transferred onto coverslips pre-coated with poly-lysine for 12 h to allow the cells to attach to the surface. The cells were exposed to a radiation dose of 2.5 Gy either alone, or in combination with 100 nM of lapatinib. The cells were then incubated for 45 min and were then washed three times with ice-cold PBS, then treated for 30 min with a 4% solution of formaldehyde in PBS for fixation, followed by incubation in 0.5% Triton X-100/PBS for 60 min, 5% bovine serum albumin (BSA) for 60 min, and a final incubation for 2 h with fluorescein isothiocyanate (FITC)-conjugated anti-phospho-Histone γ-H2AX antibody (Thermo-Fisher, USA) (1: 1500). The cells were washed with PBS and mounted in Vectashield mounting medium containing diamidino-2-phenylindole (Sigma Aldrich USA). A Zeiss LSM 8 microscope was used to examine the γ-H2AX nuclei at high power, and a mean of at least 120 nuclei was counted. The mean of the γ-H2AX foci/nuclei indicated the number of DNA double-strand breaks.

Mouse tumor xenograft model

For in vivo studies, six-week-old female C3H/HEN mice were obtained from the Animal Care Center Liaoning Cancer Hospital and Institute. The animals were maintained under controlled conditions in a laminar airflow chamber at room temperature and were fed with a normal pellet diet. The experiments were conducted at the Radiation Oncology Department of Gastrointestinal and Urinary and Musculoskeletal Cancer, Liaoning Cancer Hospital and Institute, Shenyang, China. All the animal experiments received prior approval from the Animal Ethical Committee of Liaoning Cancer Hospital and Institute, Shenyang, China, and the protocols adhered to the Animal Protection Law of the Peoples’ Republic of China (2009) for experimental animals. The approval number for the study was AIC/LCH/BC/201.

The C3H/HEN mice were inoculated with a subcutaneous injection of a suspension of MBT-2 cells (100 μl) (1×10^7 cells/100 μl) into the right flank of the mice on day 1. After one week, the tumor size was measured using vernier calipers, and the volume was calculated. A mean volume of 162 mm^3 was regarded as a criterion for tumor establishment. After successful establishment of tumor, the mice were divided into four groups: Group 1, the control group (vehicle treated with 0.5% methylene cellulose and 0.1% Tween-80); Group 2, lapatinib-treated (200 mg/kg/day); Group 3, vehicle and irradiation (15 Gy) on day 4; Group 4, lapatinib-treated (200 mg/kg/day) and irradiated (15 Gy) on the 4th day. The body weight of all the mice was recorded every week. Positron emission tomography (PET) and computed tomography (CT) scans were taken (PET/CT) by intravenous injection of the animals with 14 MBq (378 Ci) of fludeoxyglucose (FDG) in saline via the tail vein. A single animal from each group was euthanized by sodium pentobarbital anesthesia, in accordance with the guidelines for euthanasia in the Guide for the Care and Use of Laboratory Animals. The tumors were excised and fixed in 10% formalin in PBS, followed by histopathology and immunohistochemistry.

Histology and immunohistochemistry

After fixation, the tumor tissues were embedded in paraffin wax, and tissue sections were cut at 5μm using a Thermo Scientific™ HM 355S Automatic Microtome (Thermo Fisher Scientific, USA). The sections were deparaffinized using xylene, dehydrated, and microwaved for antigen retrieval. After cooling, the tumor sections were exposed to a peroxidase blocking solution. The sections were stained with hematoxylin and mounted in resin, and examined by light microscopy to identify the tumor cells. The sections were incubated with antibodies for expression of p-HER2 (InvivoGen, USA) and p-EGFR (Abcam, Cambridge, UK).

Statistical analysis

Statistical analysis was performed using GraphPad Prism™ software, version 5. The data were presented as the mean ± standard deviation (SD). Groups were compared using one-way analysis of variance (ANOVA), and for multiple comparisons, Fisher’s least significant difference (LSD) method was used. Significance was assumed to be a p-value <0.05.
Results

Lapatinib increased radiation sensitivity of MBT-2 cells

The study findings showed a dose-dependent decrease in cell survival with increasing doses of radiation ranging from 2.5–10 Gy or doses of lapatinib, which ranged from 200–1,000 nM (Figure 1A). The combination index value (CI) was calculated to determine the synergistic interaction between lapatinib and radiation on cell survival of murine mouse bladder tumor line-2 (MBT-2) cells. The dose-response data was used for the calculation of the CI. The cells treated with a 10 Gy dose of radiation and 1,000 nM of lapatinib showed CI values of <1, indicating synergy (Figure 1B).

Radiation increased the expression levels of AKT, EGFR and HER2 protein

It has previously been reported that levels of erb-B receptor family proteins were increased by radiation [9]. Previously published studies have also shown that triggering the phosphatidylinositol-3- kinase (PI3 K)/AKT cascade is linked to resistance to radiation by tumor cells [9]. In the present study, the cells received irradiation of between 2.5–10 Gy and expression of p-AKT, HER-2 and EGFR were measured at 2, 6, 12, and 24 h for both treatments (Figure 2). The results of protein expression by immunoblotting studies showed that the expression of p-AKT, HER-2 and EGFR increased in a time-dependent manner. The protein expression was normalized against the loading control.

Lapatinib suppressed radiation-induced phosphorylation of AKT, EGFR, and HER-2 in MBT-2 cells

Previously published studies have confirmed that irradiation-induced expression of erb-B receptor family proteins. In this study, the effects of lapatinib treatment were shown to down-regulate the expression of these proteins in cells subjected to irradiation alone or in combination. The cells received pre-treatment with lapatinib (100 nM and 200 nM) for 24 h and then were subjected to radiation doses of 2.5 Gy and 10 Gy. The expression of proteins was measured by Western blot, which showed that in cells treated with radiation, with or without lapatinib, the elevated levels of HER-2, EGFR, and p-AKT were down-regulated by lapatinib at 6 h (Figure 3). The protein expression was normalized against the loading control.

Lapatinib in combination with radiation enhanced apoptosis in MBT-2 cells

The outcomes of cell cycle distribution analysis after 6 h of radiation exposure of 10 Gy to cells pretreated or untreated with lapatinib (100 nM) for 30 min showed that pre-treatment with lapatinib resulted in a significant increase in sub-G1 population (p<0.05), confirming apoptosis in MBT-2 cells (Figure 4A). Cells treated with radiation alone did not show a significant rise in the sub-G1 population, but did show a decreased population of cells in the S-phase and increased numbers of cells in G2/M phase arrest. These results suggested that lapatinib treatment alone was ineffective in causing significant alterations in the phases of the cell cycle. Also, the study of protein
Figure 2. Radiation treatment increased the levels of phosphorylated epidermal growth factor receptor (p-EGFR), phosphorylated AKT (p-AKT), and phosphorylated HER-2 (p-HER2) at low and high doses in mouse bladder tumor line-2 (MBT-2) cells in a time-dependent manner. MBT-2 cells were exposed to radiation doses ranging from 2.5–10 Gy, followed by preparation of cell lysates. Western blot analysis shows a time-dependent increase in phosphorylated epidermal growth factor receptor (p-EGFR), phosphorylated AKT (p-AKT), and phosphorylated HER-2 (p-HER2).

Figure 3. Lapatinib treatment down-regulated the radiation-mediated levels of phosphorylated epidermal growth factor receptor (p-EGFR), phosphorylated AKT (p-AKT), and phosphorylated HER-2 (p-HER2) in mouse bladder tumor line-2 (MBT-2) cells in a dose-dependent manner. Pretreatment of mouse bladder tumor line-2 (MBT-2) cells with lapatinib (100 nM and 200 nM) for 24 h followed by radiation of varying doses range from 2.5–10 Gy. The cell lysates were obtained after 2 h. Western blots were performed for phosphorylated epidermal growth factor receptor (p-EGFR), phosphorylated AKT (p-AKT), and phosphorylated HER-2 (p-HER2). β-actin was used as loading control. Lapatinib treatment down-regulated the radiation-mediated levels of p-EGFR, p-AKT, p-HER2 in MBT-2 cells in a dose-dependent manner.
expression by Western blot showed that cells receiving pre-treatment of lapatinib showed upregulation of the pre-apoptotic marker, cleaved PARP when compared with untreated cells (Figure 4B). The protein expression was normalized against the loading control.

**Lapatinib, in combination with radiation, enhanced DNA damage in MBT-2 cells**

The results of immunofluorescence staining showed the presence of γ-H2AX, which is a marker of damage to double-strand DNA (Figure 5A). The control group of cells showed the complete absence or least number of γ-H2AX foci (0±0.25/cell). However, in MBT-2 cells pretreated with lapatinib and exposed to radiation, there was a significant increase in the number of γ-H2AX foci (22.1±0.50/cell) (P<0.001) compared with cells exposed to radiation alone (14.2±0.35/cell). The results of Western blot showed a dose-dependent change in the levels of γ-H2AX in cells pretreated with lapatinib (50 and 100 nM) followed by radiation of dose 2.5 and 10 Gy (Figure 5B).

**Figure 4.** Lapatinib treatment increased radiation-mediated apoptosis in mouse bladder tumor line-2 (MBT-2) cells. (A) MBT-2 cells pre-treated with lapatinib (200 nM) for 30 min followed by radiation at a dose of 10 Gy. The cell cycle distribution studies were done 6 h after cell treatment with lapatinib, and radiation in combination with lapatinib. Data presented is the mean (n=3) ±SD. * P<0.05. (B) MBT-2 cells pre-treated with lapatinib (50 nM and 100 nM) for 30 min followed by radiation at doses between 2.5–10 Gy. After 6 h, cell lysates were prepared. Western blot analysis was performed to detect the presence of the apoptotic marker, poly (ADP-ribose) polymerase (PARP).

**Figure 5.** Lapatinib increased radiation-mediated DNA damage in radiation-exposed mouse bladder tumor line-2 (MBT-2) cells. (A) The γ-H2AX count, representing radiation-induced DNA damage, was performed for 150 cells/group. The results are represented as the mean number of foci or cells per group with lapatinib (50 nM and 100 nM) for 30 min followed by radiation at doses between 2.5–10 Gy. (B) The MBT-2 cells received pretreatment with lapatinib (50 nM and 100 nM) for 30 min followed by radiation at doses between 2.5–10 Gy. The cell lysates underwent Western blot to detect phosphorylated γ-H2AX (p-γ-H2AX) and H2AX.
The combination of lapatinib with radiation therapy suppressed the growth of MBT-2 xenograft tumors in mice

The outcomes of lapatinib treatment of tumor xenografts in this animal model showed that a daily dose of lapatinib (oral, 200 mg/kg/day) for seven days, combined with radiation on the fourth day caused a significant suppression in the growth of xenografts tumors compared with irradiation alone (Figure 6A). However, oral lapatinib treatment alone had minimal effect. The results suggested that an oral dose of lapatinib increased the radiation-mediated suppression of xenografts tumors by about 60%. The results of immunohistochemistry for expression of HER-2 and EGFR in tumors recovered from mice at the end of treatment protocol of seven days showed the involvement of radiation in enhancing the levels of EGFR and HER-2 (Figure 6B). However, lapatinib, in combination with radiation therapy, suppressed the radiation-mediated activation of EGFR and HER-2 in xenograft tumors. The outcomes of this in vivo experiment indicated that lapatinib induced radiosensitization by inhibiting the radiation-mediated expression of EGFR and HER-2, in addition to facilitating DNA damage.

Discussion

The aim of this study was to evaluate the effect of lapatinib, a dual inhibitor of epidermal growth factor receptor (EGFR) and HER-2, on the radiosensitivity of murine bladder tumor line-2 (MBT-2) cells in vitro and in vivo. Lapatinib was shown to have a radiosensitizing effect on bladder tumor cells in both in vivo and in vitro models. The results of the protein expression studies showed that radiation activated phosphorylation of EGFR and HER-2.
activated the phosphatidylinositol-3-kinase (PI3K)/AKT cascade in the murine mouse bladder tumor line-2 (MBT-2) cells, which were findings supported by previously published reports [9]. The findings showed that lapatinib inhibited the radiation-induced phosphorylation of EGFR and the PI3K/AKT cascade in vitro in MBT-2 cells, and also showed that pre-treatment of the tumor cells with lapatinib enhanced radiation-induced apoptosis in MBT-2 cells. The results of cell cycle analysis suggested that pre-treatment of lapatinib followed by radiation resulted in a significant increase in sub-G1 population confirming apoptosis in MBT-2 cells (Figure 4A) when compared with cells treated with radiation or lapatinib alone. The synergistic effect of lapatinib with radiation was mediated through increased radiation-mediated DNA damage and apoptosis. The findings of this study also showed that lapatinib down-regulated the radiation-mediated HER-2 and EGFR pathways.

Lapatinib has been previously shown to have radiosensitizing effects in some breast cancer cell lines and patients with breast cancer [13–16]. To our knowledge, the present study is the first to report the effects of lapatinib in enhancing the radiosensitivity of bladder cancer cells in both an in vivo and in vitro model, although previous studies have shown that erlotinib [17] and gefitinib [18,19] have been studied in combination with radiation treatment for bladder cancer. In the present study, the murine MBT-2 cell line was studied, which is a cell line is derived from C3H/HeN mice with induced bladder tumors. The MBT-2 mouse bladder tumors resemble human bladder cancer histologically and are also reported to be immune competent [20,21], which were reasons for the selection of MBT-2 cell lines for in vivo studies.

Also, γ-H2AX is reported to be a marker of breaks in double-strand DNA [22]. An increased number of γ-H2AX foci are reported in radiosensitization studies involving EGFR inhibitors [23], which is associated with cell death [24]. The findings of this study showed that the radiosensitization effect of lapatinib was mediated by DNA damage, as shown by the presence of increased γ-H2AX foci. In a previously published study, the alkaline comet assay, a method used to identify DNA strand breakage, has been reported to assess cell survival and radiosensitivity in bladder cancer cell lines [25]. The results of the present study showed reduced cell survival and an increased number of γ-H2AX foci after combined radiation and lapatinib treatment, which indicated DNA damage. Because DNA is the key cellular target for radiation treatment of tumors, these findings support the role of lapatinib as a radiosensitizing agent for MBT-2 cells. Although these findings of this study supported that lapatinib treatment resulted in inhibition of radiation-mediated p-AKT via the HER-2 and EGFR pathway, and that combined lapatinib and radiation resulted in DNA damage, further studies are required to investigate the mechanisms involved in DNA damage in tumor cells.

Conclusions

This study evaluated the effect of lapatinib, a dual inhibitor of epidermal growth factor receptor (EGFR) and HER-2, on the radiosensitivity of murine mouse bladder tumor line-2 (MBT-2) cells in vitro and in vivo. The findings showed that lapatinib treatment radiosensitized the tumor cells by reducing radiation-induced EGFR and HER-2 activation, causing DNA damage leading to tumor cell apoptosis. These findings support a potential role for lapatinib in promoting the effects of radiation therapy, and possibly chemotheraphy, in bladder cancer.

References:

1. Boyle P, Levin B (eds.): International Agency for Research on Cancer. World Cancer Report; 2008. Available at: http://www.iarc.fr/en/publications/pdfs-online/wcr/2008/
2. Siegel R, Naishadham D, Jemal A: Cancer statistics, 2012. Cancer J Clin, 2012; 62(1): 10–29
3. Stenzl A, Cowan NC, De Santis M et al: Treatment of muscleinvasive and metastatic bladder cancer: Update of the EAU guidelines. Eur Urol, 2011; 59(6): 1009–18
4. Rodel C, Grabenbauer GG, Kuhn R et al: Combined-modality treatment and selective organ preservation in invasive bladder cancer: Long-term results. J Clin Oncol, 2002; 20(14): 3061–71
5. Coppin CM, Gospodarowicz MK, James K et al: Improved local control of invasive bladder cancer by concurrent cisplatin and preoperative or definitive radiation. The National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol, 1996; 14(11): 2901–7
6. Begg AC, Stewart FA, Vens C: Strategies to improve radiotherapy with targeted drugs. Nat Rev Cancer, 2011; 11(4): 239–53
7. Bonner JA, Harari PM, Giralt J et al: Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med, 2006; 354(6): 567–78
8. Colquhoun AJ, McHugh LA, Tulchinsky E et al: Combination treatment with ionising radiation and gefitinib (‘Iressa’, ZD1839), an epidermal growth factor receptor (EGFR) inhibitor, significantly inhibits bladder cancer cell growth in vitro and in vivo. J Radiat Res (Tokyo), 2007; 48(5): 351–60
9. Bowers G, Reardon D, Hewitt T et al: The relative role of ErbB-1-4 receptor tyrosine kinases in radiation signal transduction responses of human carcinoma cells. Oncogene, 2001; 20(11): 1388–97
10. Lipponen P, Eskelinen M, Sjyrannen S et al: Use of immunohistochemicaly demonstrated c-erb B-2 oncoprotein expression as a prognostic factor in transitional cell carcinoma of the urinary bladder. Eur Urol, 1991; 20(3): 238–42
11. Neal DE, Sharples L, Smith K et al: The epidermal growth factor receptor and the prognosis of bladder cancer. Cancer, 1990; 65(7): 1619–25
12. Shewchuk I, Hassell A, Wisely B et al: Binding mode of the 4-anilinoquinoiazoline class of protein kinase inhibitor: X-ray crystallographic studies of 4-anilinoquinazoline bound to cyclin-dependent kinase 2 and p38 kinase. J Med Chem, 2000; 43: 133–38
13. Sambade MJ, Kimple RJ, Camp JT et al: Lapatinib in combination with radiation diminishes tumor regrowth in HER2+ and basal-like/EGFR+ breast tumor xenografts. Int J Radiat Oncol Biol Phys, 2010; 77(2): 575–81
14. Sambade MJ, Camp JT, Kimple RJ et al: Mechanism of lapatinib-mediated radiosensitization of breast cancer cells is primarily by inhibition of the Raf > MEK > ERK mitogen-activated protein kinase cascade and radiosensitization of lapatinib-resistant cells restored by direct inhibition of MEK. Radiother Oncol, 2009; 93(3): 639–44

15. Harrington KJ, El-Hariry IA, Holford CS et al: Phase I study of lapatinib in combination with chemoradiation in patients with locally advanced squamous cell carcinoma of the head and neck. J Clin Oncol, 2009; 27(7): 1100–7

16. Abboud M, Saghir NS, Salame J, Geara FB: Complete response of brain metastases from breast cancer overexpressing Her-2/neu to radiation and concurrent lapatinib and capecitabine. Breast J, 2010; 16(6): 644–46

17. Colquhoun AJ, Sundar S, Rajjayabun PH et al: Epidermal growth factor receptor status predicts local response to radical radiotherapy in muscle-invasive bladder cancer. Clin Oncol (R Coll Radiol), 2006; 18(9): 702–9

18. Dominguez-Escrig JL, Kelly JD, Neal DE et al: Evaluation of the therapeutic potential of the epidermal growth factor receptor tyrosine kinase inhibitor gefitinib in preclinical models of bladder cancer. Clin Cancer Res, 2004; 10(14): 4874–84

19. Maddineni SB, Sangar VK, Hendry JH et al: Differential radiosensitisation by ZD1839 (Iressa), a highly selective epidermal growth factor receptor tyrosine kinase inhibitor in two related bladder cancer cell lines. Br J Cancer, 2005; 92(1): 125–30

20. Horiguchi Y, Kikuchi E, Ozu C et al: Establishment of orthotopic mouse superficial bladder tumor model for studies on intravesical treatments. Hum Cell, 2008; 21(3): 57–63

21. Blomgren H, Edsmyr F, von Stedingk U, Wasserman J: Bestatin treatment enhances the recovery of radiation induced impairments of the immunological reactivity of the blood lymphocyte population in bladder cancer patients. Biomed Pharmacother, 1986; 40(2): 50–54

22. Rogakou EP, Pilch DR, Orr AH et al: DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem, 1998; 273(10): 5858–68

23. Dittmann K, Mayer C, Rodemann HP: Inhibition of radiation induced EGFR nuclear import by C225 (Cetuximab) suppresses DNA-PK activity. Radiother Oncol, 2005; 76(2): 157–61

24. Gonzalez JE, Barquinero JF, Lee M et al: Radiosensitization induced by the anti-epidermal growth factor receptor monoclonal antibodies cetuximab and nimotuzumab in A431 cells. Cancer Biol Ther, 2012; 13(2): 71–76

25. Moneef MAL, Sherwood BT, Bowman KJ et al: Measurements using the alkaline comet assay predict bladder cancer cell radiosensitivity. Br J Cancer, 2005; 89(12): 2271–76