Review Article

Potential of Akt mediated DNA repair in radioresistance of solid tumors overexpressing erbB-PI3K-Akt pathway

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Summary: Phosphatidylinositol 3-kinase (PI3K)/Akt pathway is a key cascade downstream of several membrane-bound receptors, especially receptor tyrosine kinases such as erbB family members. This pathway is the most frequently activated pathway in human solid tumors. Akt/PKB (Protein Kinase B) members are the major kinases downstream of PI3K which are involved in a variety of cellular functions including growth, proliferation, survival, invasion, metastasis, and angiogenesis. Accumulated evidence indicates that activated Akt is a major predictive marker for the radiation response. Radiation-induced DNA double-strand break (DNA-DSB) is the prime cause of cell death induced by ionizing radiation. Preclinical *in vitro* and *in vivo* studies have shown that activated Akt1 accelerates repair of IR-induced DNA double-strand breaks (DNA-DSB) and, consequently, improves post-irradiation cell survival. Analyzing disregulations of Akt such as point mutations, gene amplification or overexpression, which result in constitutive activation of Akt might be of special importance in the context of radiotherapy outcome. Such studies as well as the mechanism(s) by which activated Akt1 regulates repair of DNA-DSB might help to combine the appropriate molecular targeting strategies with conventional radiotherapy to overcome radioresistance in solid tumors. Thus, in this review we discuss the disregulations in the components of upstream regulators of Akt as well as those specific modifications of AKT isoforms which enhance Akt activity. Likewise, mechanisms by which Akt improves post-irradiation cell survival will be reviewed. In this context, the role of Akt1 in repair of radiation-induced DNA-DSB will be discussed in more detail.

Key Words: EGFR-PI3K-Akt; DNA repair; radiation response

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Introduction

Radiotherapy is one of the major modalities of cancer treatment used to treat approximately 50% of all cancer patients with varying success. The dose of irradiation that can be given to the tumor is determined by the radiosensitivity of surrounding normal tissues (1) as well as intrinsic sensitivity/resistance of tumors. Resistance to radiotherapy can be either intrinsic radioresistance or an acquired resistance during fractionated radiotherapy. One of the molecular events by which tumors can become radioresistant is radiation-induced and potentially ligand-independent activation of signal transduction pathways such as those regulated by membrane-bound receptor tyrosine kinases (RTKs). In this context the role of epidermal growth factor receptor (EGFR) has been extensively investigated. In tumor cells activation of EGFR stimulates signal transduction pathways that ultimately promote tumor cell proliferation, survival, invasion, and angiogenesis (2,3). This leads to both chemo- and radiotherapy resistance and consequently to a poor prognosis (4-6). The pro-survival effect of EGFR is mediated by activation of various downstream signaling pathways, such as the phosphatidyl-

inositol 3-kinase (PI3K)/Akt pathway, the signal transducer and activator of transcription (STAT) pathway and the Ras-mitogen-activated protein kinase (MAPK) pathway (7-9). Among the pathways activated by RTKs, PI3K/Akt pathway is the major survival pathway in cancer cells, which is frequently upregulated in human tumors (10,11). PI3K/Akt pathway is constitutively active in tumor cells presenting mutation in one of the components of the EGFR downstream pathways, such as phosphatase and tensin homolog (PTEN), PIK3CA and RAS (12,13). Mutational activation of RAS and PI3K is accompanied with resistance to radio-/chemotherapy (14-17). Thus, the activation status of the PI3K pathway as a consequence of overexpression of RTKs or mutations of the signaling components might be a predictive marker for the response of tumor cells to radiotherapy. Akt, which is also known as protein kinase B (PKB), is a central transducer protein downstream of PI3K and is involved in a number of important cellular processes including cell growth, proliferation, survival, invasion, metastasis, and angiogenesis (18). In addition to these well-described functions, accumulating evidence indicates that Akt is directly involved in the control of DNA repair and radioresistance. In this review, the expression and activity status of Akt isoforms in cancers from different origins will be summarized. We also review the role of Akt family members, especially, Akt1 in radioresistance of solid tumors, and briefly summarize the role of activated Akt in the context of DNA repair.

Role of Akt/PKB in human cancers

Akt/PKB is a serine/threonine kinase, which consists of three isoforms known as Akt1 (PKBα), Akt2 (PKBβ) and Akt3 (PKBγ). These isoforms are products of different genes with more than 80% homology on chromosomes 14q32, 19q13 and 1q44, respectively (19). Different isoforms of Akt have different functions in normal physiology and development (20). Akt1 is required for normal growth (21) and mammary morphogenesis/function (22). Akt2 is essential for glucose metabolism, adipogenesis and β-cell function (23,24) and, as so far known, Akt3 is essential for attainment of normal brain size (25). For Akt over 40 downstream targets have been reported, which contribute to the cellular roles of Akt, including cell survival, growth, proliferation, angiogenesis, metabolism, and migration (26).

In human malignancies, Akt activity plays a major role in tumor cell survival (27). Enhanced activation of Akt is one of the common molecular disregulations due to AKT gene amplification, amplification-independent overexpression and hyperactivation of Akt through activation of upstream pathways (28). Hyperactivation of Akt is a common mechanism of increased cell survival, proliferation and aggressiveness in tumors presenting overexpression of Akt upstream components such as expression of a specific EGFR mutant (EGFRvIII, also known as EGFR type III, de2-7, Delta EGFR) (29-31). Mutation in RAS family members, i.e. in K-RAS and H-RAS, also stimulate Akt phosphorylation and tumor cell survival (32-36) mainly through upregulated production of EGFR-ligands such as amphiregulin and transforming growth factor α. Additionally, in tumors from different origins point mutations, loss of heterozygosity or methylation of PTEN (a phosphatase that deactivates PI3K) as one of the most frequently inactivated tumor suppressor genes (37) leads to activation of downstream components of PI3K signaling (38,39) including Akt. Immunostaining of ovarian cancer samples revealed an inverse correlation between PTEN expression and the phosphorylation level of Akt (40). Likewise, loss of PTEN is associated with worse clinical outcome as shown for patients with esophageal adenocarcinomas (41).

Previous reports indicate that amplification, mutation, or overexpression of Akt in tumors from different origins are Akt isoform-specific phenomena (28) which result in Akt hyperactivation. Hyperactivation of Akt correlates with various clinicopathologic parameters and is a prognostic indicator for cancers from different origins such as oral, pancreatic, thyroid and lung cancers (42-45). Amplification of AKT1 in general is a rare phenomenon in cancers, which was demonstrated for the first time in a primary human gastric adenocarcinoma (46) and later in a small population of estrogen receptor-positive breast carcinomas and prostate cancers (47). A somatic AKT1-E17K mutation has been reported in different cancers such as in breast; high-grade endometrial, bladder, lung and colorectal cancers (48-51). Similar to hotspot mutation, several non-hotspot mutations in AKT1 have also been identified, which cause a constitutive membrane localization of Akt1. This results in a permanent Akt1 kinase activity and phosphorylation at T308 and S473 and consequent activation of downstream target proteins independent of growth factor stimulation (52). Mutation in AKT1 (Glu17Lys) can also regulate tumorigenesis (51).

Similar to Akt1, Akt2 through amplification and overexpression exerts oncogenic activity as well. For the full activation, Akt2 needs to be phosphorylated on T309 and S474 (53). Amplification of Akt2 is more frequent

than amplification of Akt1 and has been demonstrated for human malignancies such as pancreatic, colorectal, ovarian and breast cancers (54-60). Interestingly, in ovarian cancer cells Akt1 is phosphorylated/activated and the activation/phosphorylation is Ras-, src- and PI3K-dependent when cells are stimulated with growth factors (61). Mutation analyses of AKT2 revealed that somatic mutations in AKT2 kinase domain can occur in a subset of stomach, lung, colon and breast cancers. These observations indicate that alterations in various signaling cascades depending on mutated AKT2 contribute to tumorigenesis (62).

AKT3 amplification and overexpression may play a role in the genesis of different cancers as described for a subset of ovarian cancers through modulation of cell cycle progression (63), thyroid cancer (45) and the estrogen receptor-negative breast cancers as well as androgen-insensitive prostate carcinomas (64). But so far, the most studies for the carcinogenic role of Akt3 and its impact on treatment outcome have been performed in melanoma (65,66). For the full activity, Akt3 needs to be phosphorylated at S472 and T305 (67). Similar to AKT1 (see above) an E17K mutation in AKT3 gene leads to a constitutive activity of Akt3 (66) and stimulation of substrates such as PRAS40. As reported by Madhunapantula and colleagues (68), this leads to an Akt3-dependent survival advantage and resistance to chemotherapy of melanomas (68).

Importance of Akt in radioresistance

A significant correlation between phosphorylated EGFR and phosphorylated Akt (69) indicates that EGFR is the major regulator of activation of PI3K/Akt pathway. Overexpression or mutation of EGFR has been reported in 40% to 50% of human solid tumors (70,71). Preclinical and clinical studies demonstrated that the hyperactivation of EGFR leads to both chemo- and radiotherapy resistance and consequently to a poor prognosis (4,6,31,63,72-75). Similar to ligand stimulations, exposure to ionizing radiation induces activation of EGFR and its downstream PI3K/Akt pathway (76-80). PI3K/Akt activity regulates cell growth, proliferation and survival, which will influence the response to ionizing radiation. The function of PI3K/Akt activity on radioresistance has been reported by several laboratories in different cancer cell lines including those from head and neck, colon, lung, brain cancers *in vitro* (36,76,77,80-82) independent of TP53 status (83) as well as *in vivo* (84). Although, stimulation of PI3K, in parallel to increased Akt activity, regulates the activation of other pro-survival substrates such as SGK (10), it has been demonstrated that Akt activation downstream of PI3K plays a major role in the radiation resistance of tumor cells with different entities. In this context Brognard and colleagues (85) reported that a constitutive phosphorylation of Akt, presumably Akt1, at S473 and T308 mediates radiotherapy resistance in NSCLC cells (85). In this study (85) and studies from others (34,80,84,86-89) radiosensitization was shown to be achieved either by pharmacological targeting of PI3K/Akt or genetic modulation of Akt. In a similar study conducted in bile duct cancer (BDC), Tanno *et al.* (90) described the expression of phosphorylated Akt at S473 in 84.2% of patients with BDC. Using BDC cell lines *in vitro*, these authors demonstrated that phosphorylation of Akt depends on PI3K activity (90). Targeting PI3K by LY294002 led to a similar degree of radiosensitization as observed in cells carrying a dominant-negative Akt (90). From these studies it can be concluded that Akt is a major mediator of PI3K-induced cellular resistance to radiotherapy. This conclusion is supported by reduced radiation sensitivity following overexpression of constitutively active Akt into tumor cells from different origins such as NSCLC (85), bile duct cancer (90) and colon cancer (40). In line with these preclinical findings, the prognostic value of activated Akt for the radiation response of solid tumors has been described in clinical investigations as well. Immunohistochemical analysis of phosphorylated Akt1 at S473 implies that activated Akt1 is a potential predictive biomarker for the radiotherapy response as shown for patients with head and neck cancer (91) as well as patients with locally advanced cervical cancer (92). Although, the pro-survival effect of Akt activity in oncology is well accepted, the specific mechanism by which Akt improves cell survival, especially after radiotherapy, has not been understood so far.

It has been postulated that inhibition of apoptosis is one of the mechanisms by which activated Akt improves cell survival. To inhibit apoptosis, Akt phosphorylates and inactivates pro-apoptotic proteins such as BAD, BAX and caspase-9. Akt-induced BAD phosphorylation prevents binding of BAD to BCL2 family members (93). Likewise, stimulating Akt upregulates the expression level of several intracellular antiapoptotic proteins such as FLIP, survivin, cIAP1; cIAP-2, A1/Bfl-1, and XIAPs (94,95). Akt-mediated inhibition of apoptosis abrogates the sensitivity of hematopoietic cells such as leukemia cells to therapeutic approaches inducing apoptosis (95). Ionizing radiation can also induce apoptosis through mitochondria-dependent intrinsic pathway (96). In this pathway, activated

Akt phosphorylates proapoptotic proteins BAX and BAD. Phosphorylation of Bax at S184 which is regulated in Akt-dependent manner inhibits Bax effects on the mitochondria by maintaining the protein in the cytoplasm (97). Yet, heterogeneous effects of Akt inhibition on radiation-induced apoptosis have been reported. We could show that neither in apoptosis-sensitive nor in apoptosis resistant NSCLC cell lines targeting Akt does results in enhanced radiation-induced apoptosis. In both cell types, Akt targeting led to an inhibition of repair of radiation-induced DNA-DSB and subsequent enhancement of radiation sensitivity (98). In this context it has also been demonstrated that inhibition of Akt in malignant gliomas mediates radiosensitization which is independent of apoptosis (99). Although, in a previous report by Tanno *et al*. (90), inhibition of Akt was associated with increased apoptosis, this study did not investigate impact of apoptosis on radiosensitization (90). Thus, the role of apoptosis as a mechanism of cell death in radiosensitization mediated through Akt targeting in human solid tumors is rather questionable and needs further investigations.

Experimental and clinical evidence indicates that cancer-initiating cells or cancer stem cells (CSC) are resistant to radiotherapy (100). In this context CD44 expression bears the potential to predict outcome of radiotherapy by assessment of CSC density (100). Moreover, it has been demonstrated that the expression of AKT1 and AKT2 after irradiation is increased in the breast cancer MCF-7 mammospheres CD24(-/low)/CD44(+) expressing cells, but not in the bulk population of MCF-7 CD24(+)/CD44(+) expressing cells (101). In this study (101) targeting of Akt did sensitize MCF-7 mammosphere cells, but not MCF-7 monolayer cells to ionizing radiation. Thus, it seems that Akt through a so far unknown mechanism is involved in radioresistance of CSC. The role of Akt activity in radioresistance of CSC has been demonstrated in both mammospheres *in vitro* as well as in syngeneic mice bearing tumors *in vivo* (102). Zhang *et al*. in this study (102) reported that inhibition of the Akt pathway inhibits canonical Wnt signaling as well as repair of DNA damage selectively in cancer initiating cells and sensitizes them to ionizing radiation *in vitro* and *in vivo* (102). With respect to the described functional role of PI3K/Akt pathway in CSC (99,103), inhibition of the Akt pathway might offer an improved radiation response of CSC (101,102). Based on the assumption that CSC are more radioresistant and present hyperactivation of the Akt pathway, we were able to show that selected radioresistant subpopulations of A549 cells which present the CSC marker ALDH1 can be radiosensitized by PI3K inhibitor LY294002 (104). The importance of PI3K/Akt signaling for radioresistance of CSC is also underlined by the data of Zhang *et al*. (102) indicating Akt dependence of accelerated repair of radiation-induced DNA-DSB

In recent years autophagy has been recognized as an important process in carcinogenesis as well as in processes mediating the response of tumor cells to therapy, especially radiation therapy (105,106). Clear evidence exists that PI3K/Akt signaling plays an important role in the regulation of autophagy. Exposure of tumor cells to ionizing radiation induces autophagy and previous reports indicate that inhibition of autophagy either by autophagy inhibitors (107) or genetic approaches (105) induces radiosensitization. In contrast to the cytoprotective effect of radiation-induced autophagy, induction of autophagy via the PI3K/Akt/mTOR pathway is a cytotoxic effect. This is supported by the radiosensitizing effect of Akt inhibition in malignant glioma cells through inducing autophagy (108). Further investigations are needed to identify the mechanism(s) involved in the cytoprotective effect of radiation-induced autophagy and cytotoxic effect of Akt induced autophagy on post-irradiation survival.

Control of DNA double-strand break repair by Akt as one major mechanism of radioresistance and a possible specific target for radiosensitization in tumor cells

Previous reports indicate a direct correlation between EGFR as the major regulator of Akt activity and DNA-DSB repair (74,109-111). Moreover, impact of Akt activity on DNA-DSB repair and radioresistance in tumor cells from different origins has been demonstrated as well (15,79,98,112,113). DNA-DSBs are the most lethal type of DNA lesions that lead to cell death following exposure to ionizing radiation (114). Two pathways are involved in DNA-DSBs repair, non-homologous end-joining (NHEJ) and homologous recombination (HR) (115), but NHEJ is the predominant pathway in mammals. The DNA-dependent protein kinase catalytic subunit complex (DNA-PKcs) is a major enzyme in the NHEJ process and radiotherapy response (116,117). Phosphorylation of DNA-PKcs at specific amino acid residues including T2609 cluster and S2056 are required and essential for efficient repair of DNA-DSBs during NHEJ (118). Enhanced cellular sensitivity to IR after mutations in these phosphorylation

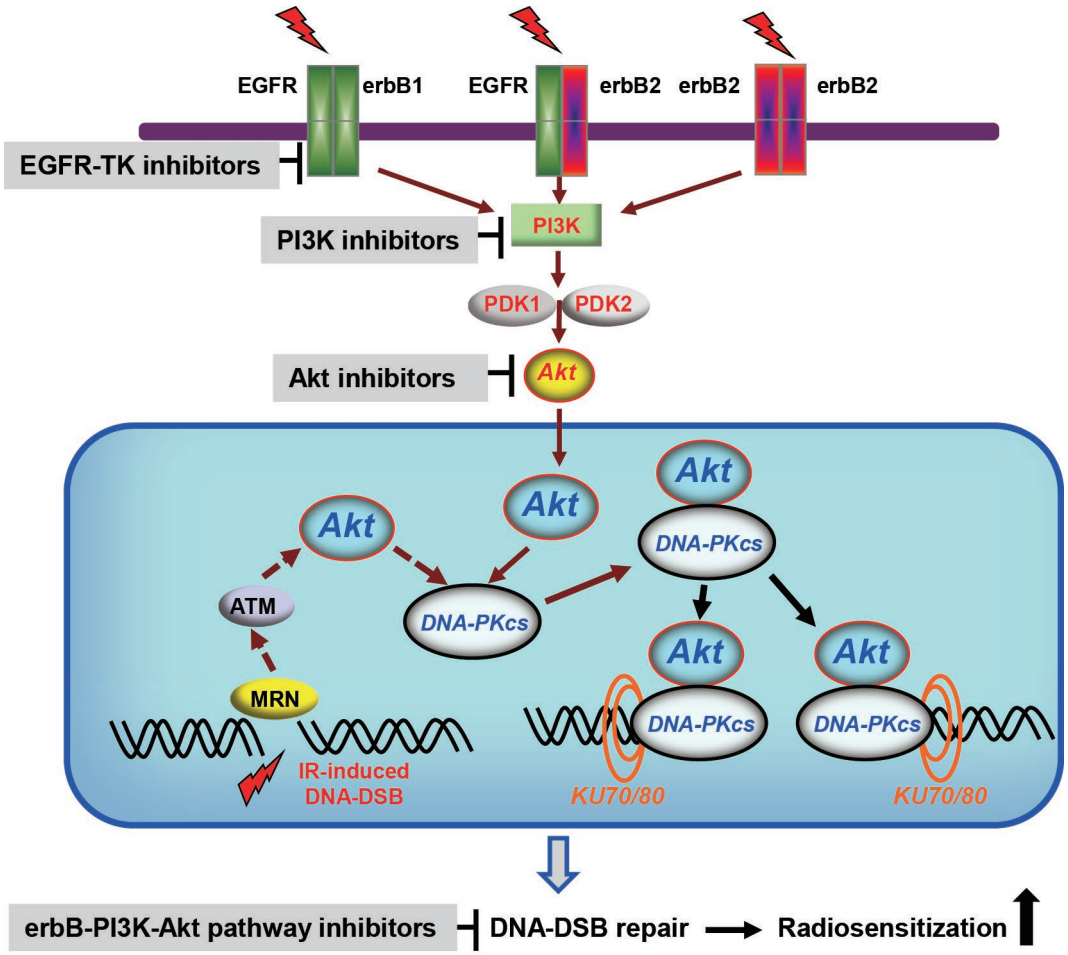

Figure 1 Illustrates the potential interaction of Akt1 and DNA-PKcs. After irradiation activated Akt translocates from the cytoplasm to the nucleus. Yet, Akt1 can also be activated in the nucleus by the MRE11-ATM pathway (113). Akt1 in the nucleus interacts and forms a functional complex with DNA-PKcs. In this complex, Akt1 stimulates DNA-PKcs kinase activity, DNA-PKcs autophosphorylation and DNA-PKcs accumulation at the DNA-double strand break. This leads to efficient repair of DNA-DSB and consequently radioresistance. Thus, inhibition of Akt activity either by targeting of receptor tyrosine kinases such as erbB receptors or targeting of PI3K as well as specific targeting of Akt can inhibit repair of radia-tion-induced DNA-DSB and improve radiotherapy outcome

sites supports the specific function of DNA-PKcs phosphorylation in DNA-DSBs repair (119-121). Previous reports from our laboratory (98,112) and the report by Park and colleagues (122) demonstrated that Akt directly interacts with DNA-PKcs through its C-terminal domain. Akt1 and DNA-PKcs form a functional complex after radiation exposure (112,123). Using GFP-tagged DNA-PKcs expressing cells, we were able to demonstrate for the first time that Akt1 promotes DNA-PKcs accumulation at the DNA-DSB site and stimulates DNA-PKcs kinase activity which is the necessary step for progression of DNA-DSB repair (112). Akt1 dependent DNA-PKcs kinase activity results in DNA-PKcs autophosphorylation at S2056 (112) which is essential for efficient repair (121) as well as the release of DNA-PKcs from the damage site. The role of Akt in DNA-DSB repair is further substantiated by co-localization γH2AX foci with P-Akt after irradiation (112,113,123,124). Based on these results, a detailed mechanism of activation of DNA-PKcs by Akt (summarized in *Figure 1*) can be proposed. Akt activity through mutation or overexpression of upstream components such as erbB receptors (30,79,109,125), PTEN (15,126), RAS (35,88,127) and PI3K (109) results in increased radiation-induced DNA-PKcs activity and enhance repair of DNA-DSB.

Thus, in the case of dysregulation of these molecules such as mutated PI3K direct targeting of PI3K or inhibition of downstream components like Akt, but not targeting of upstream components such as EGFR, can result in radiosensitization. Based on this argument, it is proposed that targeting of PI3K in combination with radiotherapy leads to heterogeneous responses depending on the Akt genotype, i.e., radiosensitization in Akt wild-type tumor cells and the lack of radiosensitization in Akt-mutated tumor cells.

An alternative pathway regulating DNA-DSB repair Akt is the upregulation of MRE11 expression after Akt activation through Akt/GSK3β/β-catenin/LEF-1 pathway (117). MRE11 is a central protein which binds to RAD50 and NBS1. It has been proven that the MRE11, RAD50 and NBS1 (MRN) complex after irradiation rapidly accumulates to damage site and remains until the damage has been repaired. MRN complex appears to be the major sensor of the breaks and subsequently recruits ATM where it is activated to phosphorylate in turn members of that complex and a variety of other proteins involved in cell-cycle control and DNA repair (128). Approximately 85% of DNA-DSBs induced by ionizing radiation are repaired within the first 2-3 h post-irradiation via the fast component of DNA-DSB repair, which has been shown to be independent of ATM function (129). The remaining 15% of DNA-DSBs, which are mainly composed of complex lesions, are repaired in an ATM-dependent manner via the slow component (130,131). Since targeting Akt leads to downregulation of MRE11 at the transcriptional level, role of Akt1 on DNA repair under this mechanism might be due to regulating slow components of DNA repair and is a complementary mechanism to Akt/DNA-PKcs dependent fast repair process. As reported by Viniegra *et al*. (132), full activation of Akt in response to insulin and ionizing radiation is ATM-dependent. In this context Fraser and colleagues (113) showed that the activation of MRE11-ATM-RNF168 pathway induce Akt phosphorylation and this leads to an Akt-dependent enhanced repair of DNA-DSB repair (113). In line with the role of ATM in Akt activity after irradiation (133), it has been reported that ATM-dependent Akt signaling regulates DNA-DSB repair in cells exposed to clinical relevant doses of irradiation (134,135). The dependence of DNA-PKcs activity through transphosphorylation at T2609 on ATM (121) as well as Akt (88,98) indicates that activation of Akt/DNA-PKcs in the nucleus can at least partially be ATM-dependent (*Figure 1*). Together, Akt-dependent activation/phosphorylation of DNA-PKcs (88,98,112) indicates that Akt is involved in the fast component of DNA-DSBs repair. Likewise, since ATM phosphorylates Akt (133) and Akt activity upregulates MRE11 (117) which is a rather slow process, it can be concluded that Akt is also part of the slow component of DNA-DSB repair. These two aspects needs to be further investigated.

In contrast to NHEJ, which in general is active throughout the cell cycle and does not rely on a template, HR is restricted to the S- and G2-phases of the cell cycle only due to the requirement for a sister chromatid as template: while the balance between both pathways is essential for genome stability, disturbance of that balance can lead to cancer (136). BRCA1, BRCA2 and RAD51 are the major proteins regulating HR repair. As described for hereditary breast and ovarian cancers, BRCA1 and BRCA2 are frequently mutated in these cancers (137,138) which might be due to genomic instability as a consequence of HR deficiency. A link between Akt1 activity and HR has also been demonstrated. HR deficiency is associated with increased phosphorylation of Akt1 at S743 as shown in breast cancer patients. Likewise, tumor formation by BRCA1 deficiency is reduced by Akt1 depletion (139). Inhibition of HR by Akt1 activity in BRCA1 proficient breast cancer cells has been demonstrated to be due to induction of cytoplasmic retention of BRCA1 and RAD51 (140). In HR-deficient cells, Akt1 mediated inhibition of HR is through impaired Chk1 nuclear localization and subsequently disruption of Chk1-Rad51 interaction (141). Thus, from these studies it becomes clear that in contrast to the stimulatory effect of Akt1 on NHEJ, in tumor cells aberrant activation of Akt inhibits HR and generates genetic instability (140). Regarding the role of Akt activity regulated by PTEN in DNA-DSB repair conflicting reports exist. Puc *et al*. (142) have demonstrated that PTEN knockdown generates DNA damage due to insufficient inactivation of CHK1 under non-irradiated basal condition. In this report by Puc *et al*. (142), although γH2AX foci assay in control-siRNA transfected and irradiated cells was used as positive control, the effect of PTEN-siRNA on residual DNA-DSB was not investigated. Thus, this study does not provide strong evidence that inhibition of HR is mediated through Akt activity. With respect to the role of PTEN in DNA-DSB repair, Kao *et al*. (15) demonstrated that overexpression of PTEN results in decreased Akt1 phosphorylation and increased residual DNA-DSB. The study and results by Kao *et al*. do not support the role of Akt activity in HR as suggested by other groups (139-141), i.e. that reduced Akt activity following PTEN overexpression leads to an increase

in HR and consequently accelerated repair of DNA-DSB. Overexpression of PTEN, associated with reduced Akt activation, resulted in an impaired repair of DNA-DSB (15). Since the effect of Akt on DNA-DSB repair is a combination of both NHEJ and HR repair pathways, it can be assumed that the effect of Akt stimulated repair of DNA-DSB by NHEJ is dominant over Akt mediated impairment of DNA-DSB repair in HR-deficient cells. A study by Li *et al*. (143) indicates that EGFR tyrosine kinase inhibitor erlotinib attenuates homologous recombinational repair of chromosomal breaks in human breast cancer cells after irradiation (143). Since erlotinib inhibits Akt phosphorylation, from this study as well as from a study by Kao *et al*. (15) it may be concluded that Akt has a differential effect on HR after irradiation on tumor cells compared to non-irradiated condition. Nevertheless, the distinct role of Akt in repair of DNA-DSB through the HR pathway needs to be further investigated.

The reports discussed in this chapter underline the role of Akt in repair of DNA-DSB via NHEJ repair process. So far, to our knowledge no clinical study combining Akt inhibitor treatment with radiotherapy has been conducted. However, data obtained for the combination of dual PI3K and mTOR inhibitors such as BEZ235 with radiotherapy in animal systems suggest that inhibition of PI3K/Akt pathway is an effective strategy to improve radiotherapy, especially in tumors with K-RAS mutation (144). This study also indicates the importance of constitutive Akt activity stimulated in K-RAS mutated cells for effective DNA-DSB repair.

With respect to the approach to use the inhibitors of NHEJ pathway to improve radiotherapy of tumors, the effect on normal tissue should not be ignored. Since the NHEJ repair pathway is also the major pathway for repair of DNA-DSB in normal cells; targeting of the components of this pathway will inhibit repair of DNA-DSB simultaneously in tumor cells as well as in normal cells. Therefore, in spite of efficient radiosensitization by using targeting of the components of NHEJ repair pathway such as DNA-PKcs, molecular targeting of NHEJ due to normal tissue damage cannot be applied to improve radiotherapy. Since dysregulations of PI3K/Akt pathway due to mutation, gene amplification or overexpression is a tumor-specific phenomenon; inhibition of Akt-dependent DNA repair pathway might be a selective approach to kill tumor cells.

Akt inhibitors are either ATP mimetics or allostric inhibitors which bind to the pleckstrin-homology domain of Akt. The current clinical trials in oncology are testing the feasibility and applicability of these inhibitors such as Perifosine, MK2206 and Nelfinavir (allosteric Akt inhibitor) or GSK690693 (ATP-competitive inhibitor) (10,145). Most of the so far described Akt inhibitors target all 3 Akt isoforms. Depending on the outcome of clinical trials in oncology with the Akt inhibitors, Akt targeting strategies, especially, targeting of Akt1 prior to radiotherapy might be highly effective to overcome PI3K/Akt-dependent radioresistance of solid tumors.

Conclusions

Molecular targeting approaches in oncology are based on understanding the function of cellular signaling pathways in tumor growth, proliferation and survival. Activation of EGFR/PI3K/Akt signaling pathway is crucial for post-irradiation cell survival. Most of the small-molecule inhibitors used for targeting signal components within this pathway are cytostatic rather than cytotoxic. Likewise, hyperactivation of the downstream components of this pathway such as mutations in PTEN, PI3K, or Akt related genes results in a lack of response to inhibitors of upstream molecules, e.g., EGFR. Although EGFR is the major regulator of the PI3K/Akt pathway, this pathway can also be regulated by many other receptors like G-protein coupled receptors, integrin receptors and insulin receptors. Thus, based on the preclinical studies combining Akt inhibitors with radiotherapy can shift the cytostatic effect of Akt inhibitors towards an increased cytotoxicity of radiotherapy. Likewise, as a consequence of targeting Akt as the key player of PI3K/Akt signaling, the crosstalk between several membrane-bound receptors cannot result in reactivation of Akt signaling.

Thus, based on the described role of Akt in repair of DNA-DSB, Akt rather than EGFR or PI3K should be the prime molecule to be targeted in order to overcome radiotherapy resistance of solid tumors presenting enhanced PI3K/Akt signaling.

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Footnote

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