Am J Cancer Res 2016;6(4):827-842
www.ajcr.us /ISSN:2156-6976/ajcr0027139

Original Article

Regulation of therapeutic resistance in cancers by receptor tyrosine kinases

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Received March 2, 2016; Accepted March 6, 2016; Epub March 15, 2016; Published April 1, 2016

Abstract: In response to DNA damage lesions due to cellular stress, DNA damage response (DDR) pathways are activated to promote cell survival and genetic stability or unrepaired lesion-induced cell death. Current cancer treatments predominantly utilize DNA damaging agents, such as irradiation and chemotherapy drugs, to inhibit cancer cell proliferation and induce cell death through the activation of DDR. However, a portion of cancer patients is reported to develop therapeutic resistance to these DDR-inducing agents. One significant resistance mechanism in cancer cells is oncogenic kinase overexpression, which promotes cell survival by enhancing DNA damage repair pathways and evading cell cycle arrest. Among the oncogenic kinases, overexpression of receptor tyrosine kinases (RTKs) is reported in many of solid tumors, and numerous clinical trials targeting RTKs are currently in progress. As the emerging trend in cancer treatment combines DNA damaging agents and RTK inhibitors, it is important to understand the substrates of RTKs relative to the DDR pathways. In addition, alteration of RTK expression and their phosphorylated substrates can serve as biomarkers to stratify patients for combination therapies. In this review, we summarize the deleterious effects of RTKs on the DDR pathways and the emerging biomarkers for personalized therapy.

Keywords: Receptor tyrosine kinase, DNA damage, DNA repair, epidermal growth factor receptor, signaling

Introduction

DNA damage stimuli can be divided into two classes, endogenous and environmental, based on the site of the stimulus' origin [1]. Endogenous DNA damage generates chemical changes in DNA structure leading to mutagenic events such as deamination of bases resulting from hydrolytic and oxidative events inside the cell. Environmental DNA damage can result from either physical or chemical agents outside the cells [1]. The incidence of DNA damage occurs frequently in normal cells. It is estimated that the error rate of the DNA replication machinery is at least 10^{-8} in *Escherichia coli* and human [2, 3]. In addition to replication errors, DNA breaks mainly caused by reactive oxygen species (ROS) are estimated to be 10^5 events per day [4, 5]. Thus, DNA damage response (DDR) is required to correct mistakes in DNA and is also responsible for eliminating cells with irreparable deleterious damage.

DNA damage and DDR are highly related to the formation and treatment of cancer. During carcinogenesis, the inefficiency and infidelity of the DDR pathway are the main causes of oncogenic events, such as DNA mutations, translocations, and epigenetic modifications, which correlate DDR to cancer risks [1, 6-9]. In cancer treatment, both radiotherapy and chemotherapy utilize DNA damaging agents that eliminate cancer cells by inducing DDR. Capitalizing on the deficiency of DDR in cancerous cells, the treatment of cancer with DNA damaging agents is an effective means of inducing massive DNA lesions and programmed cell death in the cells unable to resolve the damage. However, resistance to these types of treatment is reported in patients, and the crosstalk between DDR and

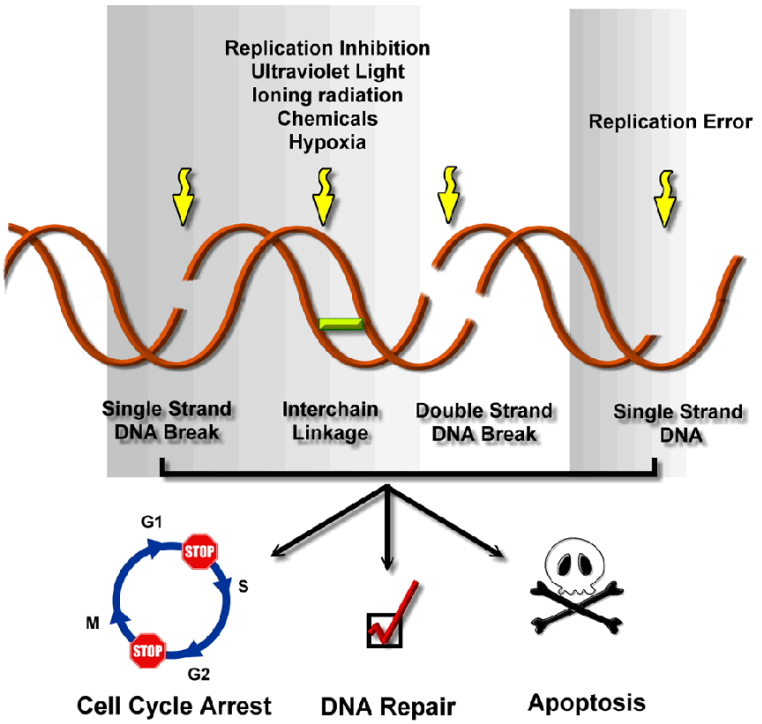

Figure 1. DNA damage reagents and DNA damage response. DNA is vulnerable to both exogenous and endogenous DNA damage reagents, including replication error, replication inhibition, ultraviolet (UV) light and cancer treatments such us irradiation therapy and chemotherapy. Exposure to these DNA damage reagents leads to DNA damage including DNA single-strand break (SSB), DNA interstrand cross-linking (ICL), DNA double-strand break (DSB) as well as single-strand DNA lesion (ssDNA). The DNA damage lesions then trigger the signal cascade which results in DDR primarily through delayed cell cycle from G1 to S phase (G1/S arrest) or from G2 to M phase (G2/M arrest) and as well as triggering DNA damage repair pathways. After successfully repaired, the cell cycle arrest is released and the cells will survive. However, the severe DNA damage adducts or DNA damage repair failure will eventually leads to apoptotic cell death.

altered receptor tyrosine kinase (RTK) signaling pathways in solid tumors is thought to be an important contributor to the development of chemotherapy resistance [10-13]. Overexpression of RTKs also contributes to tumor progression through promotion of cell survival, metastasis and stimulation of angiogenesis [14]. While many inhibitors targeting RTKs are already in clinical trials or clinical use [14], it is important to understand how RTKs promote cell survival upon DNA damage to develop combination therapies to enhance treatment efficacy.

DNA damage response

Once the DNA damage sensor protein machinery detects DNA damage lesions, it recruits mediators and numerous transducer and effector proteins to ensure that the transcription and translation processes are paused by cell cycle arrest and to initiate DNA damage repair or apoptosis (**Figure 1** and **Table 1**) [1]. The main mediators in DDR pathways are members of the phosphatidylinositol 3-kinase-like protein kinases family, including ataxia-telangiectasia mutated (ATM), ATM and Rad 3-related (ATR), and DNA-dependent protein kinase (DNA-PK). When DNA damage lesions are recognized by a sensor protein, these mediators are recruited to the damage site and phosphorylate downstream proteins that are involved in all aspects of DDR. In addition to these mediators, poly (ADP-ribose) polymerases (PARPs), a large enzyme family with multiple functions, also play important roles in DDR [15, 16]. PARP1 and PARP2 are activated by DNA single-strand break (SSB) and DNA double-strand breaks (DSB) and can poly(ADP-ribos)ylate (PARylate) different substrates under different genotoxic stress to further elevate DDR response [17].

Cell cycle arrest

The cell cycle is subdivided into G1, S, G2, and M phase. In brief, cells increase in size and prepare for DNA synthesis during G1 phase and undergo DNA replication during S phase. Then, cells continue to grow and prepare for mitosis in G2 phase before dividing in M phase. To ensure genomic stability, eukaryotic cells develop cell cycle checkpoints that pause cell division in response to environmental stress, DNA damage, and improper DNA replication [18]; this process is referred as cell cycle arrest. In mammalian cells, there are two major signaling pathways that control cell cycle arrest in response to DNA damaging stress: the ATM

Table 1. Sources of DNA damage and major repair pathways

Sources of damage	Spontaneous reactions AAs	X-ray ROS AAs 5-FU	UV Aromatic groups Hydrocarbons		Replication inhibitor UV IR Platin ROS Hypoxia				Replication errors
DNA damage types	O^6mG Pyrimidine dimer	8-oxoG AP site Uracil	CPD 6,4-P.P. DNA crosslink		DNA crosslink dsDNA break				Base mismatch Insertion Deletion
Repair pathway	Direct reverse	BER	NER		HR		NHEJ		MMR
			GG-NER	TC-NER	ATR	ATM	C-NHEJ	A-NHEJ	
Sensor/Initiator	MGMT AGT	OGG1 PARP	XPE XPC HR23B	RNA Pol I/II CSA CSB PARP	ATRIP	MRN PARP	Ku70 Ku80	PARP	MSH2 MSH3 MLH1
Transducer/Effector		XRCC1 APE1/2	RPA XPA XPC TFIIH XPB XPD XPG XPF ERCC1		ATR Chk1	ATM Chk2	DNA-PK WRN Artemis XRCC4 XLF	Fan1 PNKP XRCC1	
					MRN Rad51 BRCA 1/2 XRCC 2/3 P53 Rad52 Rad54				
Elongation/Ligase		PCNA DNA Polβ Lig III	PCNA RFC FEN1 Lig I DNA Pol δ/ε		PCNA Lig I DNA Pol δ/ε		DNA Polβ Lig IV	Lig III	RFC PCNA EXO1 DNA Pol δ Lig I/IV

Abbreviations: AAs, alkylating agents; ROS, reactive oxygen species; 5-FU, 5-Fluorouracil; IR, ioninzing radiation; O^6mG, O6-methylguanine; 8-oxoG, 8-oxoguanine; CPD, cyclo-butane pyrimidine dimer; 6,4-P.P, 6-4 photoproduct.

pathway, which is responsible for DSB throughout the cell division cycle and the ATR pathway, which is responsible for both DSB as well as replication forks [18, 19].

DNA damage repair and therapeutic DNA damaging agents

In 1974, researchers had already realized that the integrity of DNA is vulnerable and that the repair mechanisms are crucial to maintain genomic stability. Dr. Francis Crick stated in *The double helix: a personal view* that "... one could hardly discuss mutation without considering repair at the same time" [20]. The DNA damage repair pathways are composed of base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), single-strand annealing (SSA), homologous recombination repair (HR) and non-homologous end joining repair (NHEJ) [1].

Repair of base alternation and small DNA damage adducts

Base alternation and small DNA damage adducts, covalent DNA-chemical binding structures, can be caused by low concentration of reactive oxygen species (ROS) as well as alkylation agents and DNA crosslinking agents. Small DNA damage adducts can be easily repaired in normal cells compared with DSBs, but the failure to repair these adducts' fidelity may lead to oncogenic mutations. In cancer treatment, low doses of ionizing radiation (IR) and low linear energy transfer γ-radiation [21] can generate low concentrations of ROS whereas a large number of chemotherapeutic drugs are alkylating agents, including nitrogen mustards (mechlorethamine, cyclophosphamide, and ifosfamide), nitrosourease (streptozocin, carmustine and lomustine), alkyl sulfonates (busulfan), triazine (dacarbazine and temozolomide) and ehylenimines (thiotepa and altretamine) [22].

Base excision repair: BER is mainly responsible for small lesions caused by endogenous DNA damage, such as oxidation, hydroxylation, deamination, or methylation, and is considered to be the most frequently used DNA damage repair pathway [1, 23]. Abnormal DNA bases are detected and excised by lesion-specific DNA glycosylases, such as OGG1 and MYH, creating apurinic, apyrimidinic, or abasic sites (AP sites) [1]. For AP sites limited to a single base, the short patch BER endonuclease APE1 generates a single nucleotide gap at the AP site and recruits DNA polymerase β as well as XRCC1-DNA ligase to fill the gap. For extensive AP sites (2-10 bases), long patch BER with FEN1 endonuclease and proliferating cell nuclear antigen (PCNA)-DNA polymerase δ/ε complex are used to repair the lesions [1]. Genetic variants of ADPART, XRCC1, APE1 proteins in BER are reported to increase the risk of squamous cell carcinoma [24, 25] and bladder cancer [26]. APE1 and XRCC1 polymorphisms have been reported to correlate with gastric cancer [27] and with risk of lung adenocarcinoma [28, 29], respectively. The nitrogen (*N*-) and oxygen (*O*-) alkylated DNA bases caused by alkylating agents as well as oxidative DNA bases induced by ROS are repaired by BER [30].

PARP participates in many DNA repair pathways including BER, NER, HR and NHEJ [31, 32] but predominantly functions in the BER pathway. Although PARP is not essential in the BER pathway, the treatment of PARP inhibitor has successfully converted the base lesion into a SSB [33], which is a more severe type of DNA damage that can be developed into lethal DSB lesions during DNA replication [15, 34]. PARP inhibitors, for example, olaparib, can induce synthetic lethality in DSB repair-deficient cancer cells, such as *BRCA*-mutated cells, and benefit patients with *BRCA1/2*-mutated breast or ovarian cancer [15, 35-37].

Nucleotide excision repair: NER mainly tackles a variety of helix-distorting lesions that impede transcription and replication by interfering with base pairing [1, 23]. Global genome NER (GG-NER) repairs helix-distorting lesions and prevents mutagenesis. Transcription-coupled NER (TC-NER) repairs transcription-blocking lesions to prevent perturbed gene transcription. The damage recognition steps are different between these NER mechanisms: in GG-NER, the lesions are detected by XPC/hHR23B and XPE protein complex, whereas the RNA polymerase/CSA/CSB/HMGN1 protein complex is responsible for lesion detection in TC-NER [1]. After lesion recognition, XPA proteins are recruited and bind to DNA around 20 base pair upstream of the DNA damage adduct. The DNA double helix around the DNA damage adduct is then unwound by a multi-protein complex, TFIIH. Single-stranded DNA (ssDNA) res-

ulting from the unwinding process is stabilized by the RPA protein, and the DNA adduct excision steps are completed by the XPF-ERCC1 and XPG proteins. NER repairs DNA lesions caused by various endogenous and environmental DNA damaging agents, including UV irradiation [38], platin-based chemotherapy drugs, e.g., cisplatin and carboplatin [39], and carcinogens, such as benzopyrene [40]. Defects in NER result in diseases, such as xeroderma pigmentosum (XP), Cockayne syndrome, and trichothiodystrophy [1]. Among the NER-related diseases, XP patients, but not Cockayne syndrome or trichothiodystrophy patients, exhibit a higher incidence of skin cancer. For example, XP group A patients, a subpopulation of XP patients, are more prone to basal cell and squamous cell carcinoma, and melanoma [41, 42].

Mismatch repair: MMR is designed to resolve mispaired or modified bases as well as insertion or deletion loops. Heterodimers of the MSH2/MSH6 complex recognize mismatched pairs and single-base loops whereas the MSH2/MSH3 complex recognizes insertion/deletion loops. This damage recognition complex then recruits and interacts with MLH1/PMS2 and EXO1 endonuclease to excise the newly synthesized strand after mismatch/loop. DNA is then resynthesized by PCNA, RPA, and DNA polymerase δ/ε complex [1]. Germline mutations in MLH1 and MSH2 have been shown to contribute to hereditary non-polyposis colorectal cancer [43], and defects in MSH6 are known to cause atypical hereditary non-polyposis colorectal cancer. Germline variants in DNA polymerase ε are also associated with MMR-deficient colorectal cancer [44]. MMR deficiency testing can predict the prognosis of colorectal cancer and stratify patients for adjuvant chemotherapy [45].

Repair of DNA double-strand breaks

DSBs are considered to be lethal DNA damage lesions that must be repaired before cell continues to grow and proliferate. Currently, radiotherapy and most chemotherapies aim at creating irreparable DSBs in cancerous cells. In cancer treatments, radiotherapies, such as ionizing radiation, induce high concentrations of ROS [21]. Topoisomerase poisons, such as doxorubicin and daunorubicin, can cause DSBs [46].

Non-homologous end joining: NHEJ is important for DSB repair in DNA damage repair as well as for V(D)J recombination in T and B cells [47]. NHEJ functions in DSB repair throughout the cell cycle, especially in G0/G1 phase, and is highly conserved from prokaryotes to eukaryotes, demonstrating its mechanistic flexibility and tolerance for various structures of DNA ends [48-50]. NHEJ is a highly mutagenic repair pathway in that it ligates two ends at the DSB site together regardless of the homology of the DNA sequence [50]. NHEJ can be divided into two pathways, canonical NHEJ (C-NHEJ) and alternative NHEJ (A-NHEJ), according to the resection of DNA ends at the breakage site and the proteins involved [47]. For C-NHEJ, Ku proteins bind to the broken ends of DSBs and recruit DAN-PK as well as 53BP1 and the Mre11 complex to the damage site. The breakage sites are then processed by Artemis and are simply ligated in *cis* by the XRCC4/Ligase IV/XLF complex [47]. The direct ligation process in C-NHEJ alters the DNA sequence at the damage site, resulting in more mutations as extra nucleotides are excised before ligation. For A-NHEJ, the DNA breakage ends are recognized by PARP1, which recruits the Mre11 complex to the damage site before a few nucleotides are excised by CtIP-mediated end resection. The gap can then be filled and ligated by the XRCC1/Ligase III/Ligase I complex [47].

Homologous recombination repair: HR repair is the predominant type of DSB repair that occurs in late S and G2 phases of the cell cycle [51]. The HR pathway utilizes a DNA template strand with significant sequence homology to the damaged strand; therefore, this repair pathway is considered to be error-free and non-mutagenic [1]. The regulation and flexibility of the Mre11 nuclease activities are important in controlling the repair pathway choice during DSB repair [52]. The HR pathway initiates binding of the Mre11-Rad50-Nbs1 protein complex (MRN) to the DSB site and the cyclin-dependent kinase (CDK)-dependent activation of the CtIP protein, which regulates Mre11-mediated end resection of DNA [52, 53]. After initial resection, the Exo1-DNA2-Sgs1 complex is responsible for further DNA resection, and the ssDNA is protected by the RPA proteins [51]. The RPA proteins are then replaced by Rad51 in a BRCA1/BRCA2 dependent strand invasion process, and the pairing of homologous sequence is completed

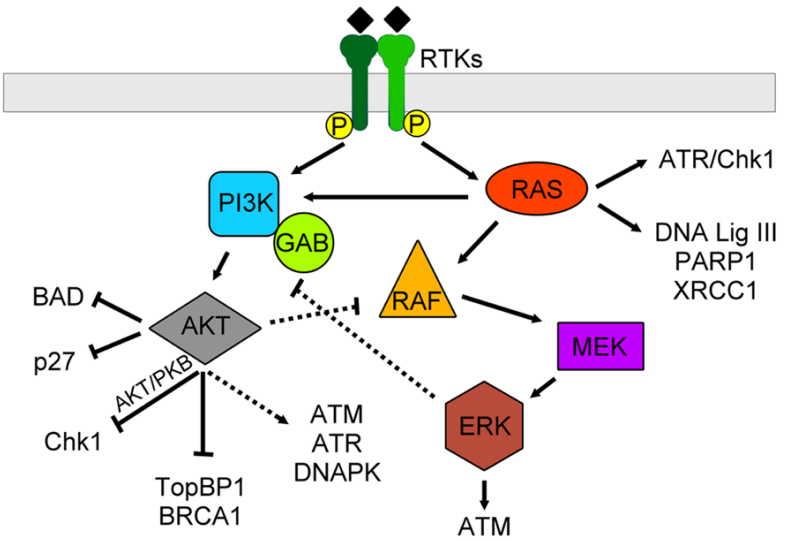

Figure 2. RTKs mediate DDR through canonical AKT and RAS pathways. In general, RTKs can activate both AKT and RAS pathways. Crosstalk between these two pathways can occur through AKT-RAF and ERK-GAB interactions. The downstream effects of the AKT pathway include inhibition of apoptosis through BAD and p27, inhibition of cell cycle progression through Chk1, downregulation of DNA damage repair through BRCA1, and indirect upregulation of DNA damage repair through ATM, ATR and DNAPK. Meanwhile, RAS itself can activate cell cycle arrest through ATR and Chk1 while promote DNA damage repair through expression of DNA ligase III, PARP1 and XRCC1. Also, the downstream of RAS pathway can activate ATM to promote DDR.

and extended with the help of Rad52, Rad54 and WRN complex proteins [54]. The junctions at homologous pairing site are then resolved by the BLM/TOPIII/Mus81 complex [54].

Cancer cells are highly proliferative and divide more frequently than cells in normal tissue. Genomic integrity in S and G2 phase is required before cells divide; therefore, inhibiting HR and initiating DSB in HR-deficient cells are both efficient ways to inhibit cancer cell proliferation by trapping cells in the G2/M cell cycle checkpoint. Chemicals that serve as HR inhibitors are often involved in regulating protein expression, nuclear localization, and recruitment of HR proteins. For example, inhibitors of histone deacetylation and HSP90 can block HR by diminishing the expression of BRCA2 [55] and Rad51 [56]. There are also cancer cells that have HR-deficiency. For example, *BRCA1/2* germline mutations are reported in many patients with solid tumors, especially in hereditary breast and ovarian cancer patients [57, 58]. The deficiency of HR leads to sensitization of patients to DNA damaging agents such as cisplatin and PARP1 inhibitors [59-63].

Single-strand annealing: SSA is an error prone repair mechanism that is initiated when DSBs occur between two repeated intra-strand DNA sequences. The ERCC1/XPF complex is responsible for the DNA excision step in SSA [64]. After excision of the 5'-ends and exposing regions of homology, the homologous strands of DNA must be paired through SSA, as in HR. Unlike HR, the RAD52 and RAD59 proteins play a predominant role in the SSA DNA binding step instead of RAD51 [65]. SSA is reduced in G1-arrested cells, but it is not clear whether the SSA pathway is cell cycle dependent because it is not under control of ATM, ATR, or DNA-PK [64]. Although SSA utilizes homologous pairing of repeated DNA sequence, it excises the repeated sequence closer to the site of the DNA break and could be mutagenic. However, the detailed mechanism and regulation of SSA is still unclear. Therefore, the importance of SSA in cancer formation and progression cannot be clearly addressed at this point in time.

RTK signaling

RTKs are highly conserved in protein structure, activation mechanisms, and downstream regulations from *C. elegans* to humans [66]. In human, there are 20 RTK subfamilies composed of 58 known RTKs [67], which activate both the canonical and non-canonical signaling pathways [67]. In the canonical signaling cascade, RTKs are localized at the cell surface membrane where they are activated by ligands from outside of the cell. Subsequently, they recruit downstream substrate proteins to the membrane, and the substrate proteins are phosphorylated to recruit more proteins to transduce the signaling cascade in the cell. In non-canonical RTK signaling pathways, the RTKs are internalized into the cells after activation and translocate from cell surface into the

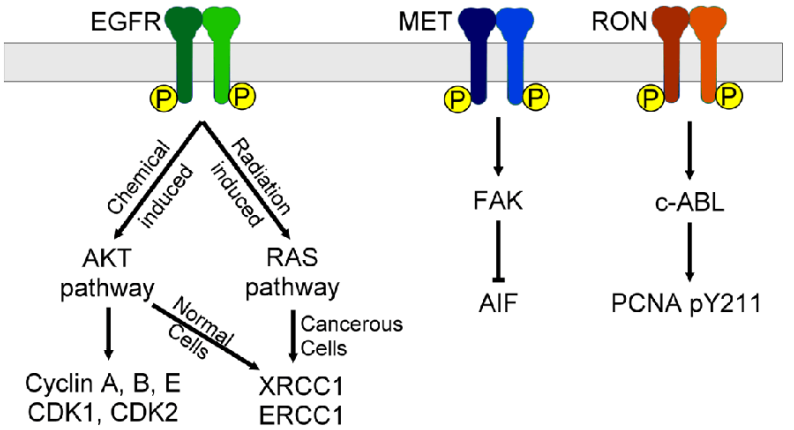

Figure 3. Some RTKs regulate DDR through specific canonical pathways. The chemical-induced EGFR activation will activate AKT pathway to upregulate the expression of cyclin A, B, E and CDK 1, 2 to promote cell cycle. On the other hand, the radiation-induced EGFR activation will activate RAS pathways to increase expression of XRCC1 and ERCC1 in cancer cells. MET can downregulate the expression of AIF specifically through the FAK pathway. Ron promotes PCNA Y211 phosphorylation through c-ABL mediated pathway.

cytosol and nucleus where they phosphorylate their substrates [67].

Canonical RTK signaling cascade

For the canonical signaling cascade, receptors are initially activated through conformational changes induced by ligand binding, and their kinase activity is further elevated through receptor oligomerization [68]. These *trans* auto-phosphorylated tyrosine sites then serve as docking sites for downstream cytosolic adaptor proteins containing phosphotyrosine-binding and Src homology-2 (SH2) domains [69]. Different adaptor proteins trigger different signaling pathways, including the RAS and PI3K protein-mediated pathways. In the RAS-mediated pathway, Grb-2 protein serves as an adaptor protein that binds to and is activated by phospho-RTK before recruiting and activating the RAS protein. The activated RAS protein then triggers the signaling cascade comprised of RAF, MEK and ERK proteins to enhance cell proliferation and transformation through regulating transcription factors and cell cycle regulatory proteins, such as AP1 and cyclin D1 [70, 71]. In the PI3K-mediated pathway, the p85 regulatory subunit of PI3K serves as an adaptor protein to the activated RTK, and the p110 catalytic subunit of PI3K further phosphorylates downstream proteins, such as protein kinase C (PKC) and AKT [72, 73]. The activated PKC signaling pathways promote proliferation, survival and metastasis of potential of cancer cells [74, 75]; activated AKT signaling pathways also promote cell proliferation and survival [76]. Canonical RTK signaling is featured by its signaling redundancy among RTKs and the signaling crosstalk among the downstream pathways. Although RTKs can regulate common downstream proteins, these signaling pathways also feature signaling crosstalk among different parallel pathways. One RTK can transactivate other RTKs to regulate signaling cascade. For example, insulin-like growth factor (IGF-1)-induced IGF-1 receptor (IGF-1R) activation can also transactivate EGFR by promoting protease dependent-release of EGFR ligand. Transactivated EGFR accounts for the majority of RAS signaling induced by IGF [77]. RAS stimulates PI3K activity whereas AKT inhibits RAF activity. Both RAS-mediated and AKT-mediated signaling pathways can regulate common downstream proteins, such as mTOR [78]. Although the RTK signal redundancy and crosstalk suggest that downstream signaling proteins may be better targets than RTK itself, inhibitors blocking these downstream signaling proteins can affect both normal and cancer cells. To minimize the cytotoxicity effect on normal cells, targeting cancer cell-specific mutated protein and cancer cell-addicted RTK may be more practical.

Non-canonical RTK signaling pathway

In canonical RTK signaling, the activated receptors are internalized and are either recycled to the cell surface or subjected to lysosomal degradation. Although the majority of internalized RTKs are recycled [79], not all of the other internalized RTKs are degraded. A small portion of internalized RTKs, for example, about two percent of internalized EGFR, can phosphorylate non-canonical cytosolic and nuclear substrates and can trigger non-canonical RTK signaling pathways [67, 80]. The first nuclear localized

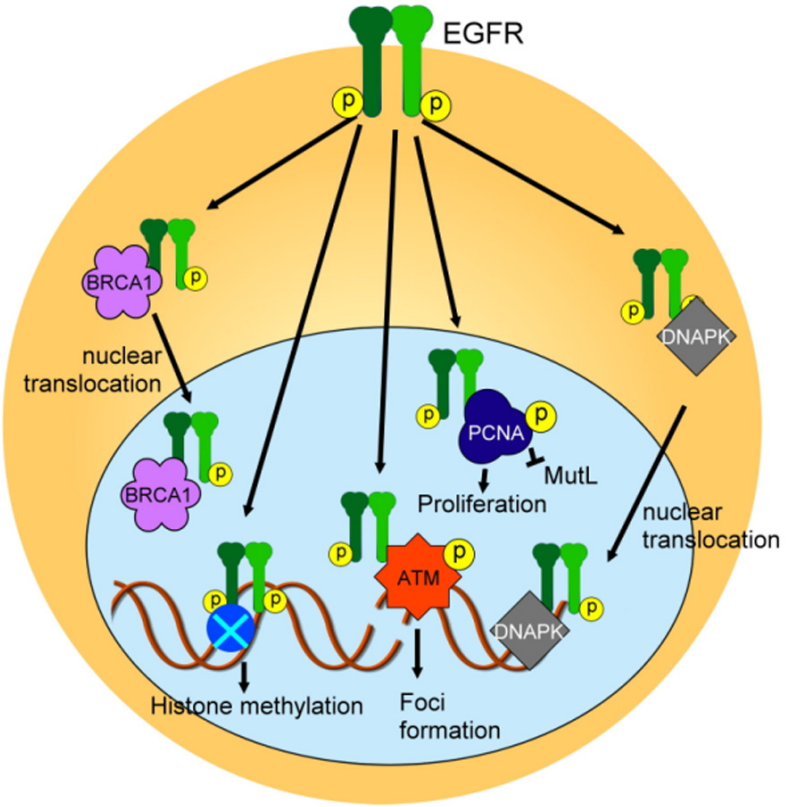

Figure 4. EGFR also mediates DDR through non-canonical signaling pathways. EGFR interacts with BRCA1 and DNAPK to promote their translocation into the nucleus. EGFR can also phosphorylate histone, ATM and PCNA to promote histone methylation, foci formation, and proliferation whereas EGFR-mediated PCNA phosphorylation inhibits MutL activity.

RTK was discovered in 1984 [81], and the non-canonical functions of nuclear localized RTKs were characterized by 1994 [82]. To date, more than 14 RTK subfamilies have been reported to play an important role in promoting cell proliferation, survival, DNA repair and drug resistance through non-canonical nuclear signaling pathways [67]. For instance, activated EGFR and HER2 are internalized into the cell and translocates into nucleus through retrograde trafficking mechanisms to endoplasmic reticulum (ER) before translocate from ER to nucleus through membrane-bound trafficking mechanism known as integral trafficking from the (ER) to the nuclear envelope transport (INTERNET) pathway [67, 83-85]. Nuclear EGFR then serves as a transcription co-activator to promote proliferation, inflammation, survival, and drug resistance by interacting with transcription factors, e.g., E2F1, STAT3/5, and RHA, enhancing the expression of proteins such as cyclin D1, iNOS, c-Myc, B-Myb and BCRP [86-91]. Nuclear EGFR also functions as signaling transduction kinase to promote proliferation, DNA repair, and cell survival by phosphorylating PCNA, Histone H4, ATM, and DNA-PK [10, 80, 92-95]. On the other hand, fibroblast growth factor receptor (FGFR) is reported to transport to ER via a retrograde pathway and then translocate from ER into the nucleus in a membrane-vesicle independent mechanism which is a part of integrative nuclear FGFR-1 signaling (INFS) [67]. Nuclear FGFR then serves as transcription regulator by interacting with CREB-binding protein and STAT5 [96].

Regulation of RTK signaling on DDR and therapeutic resistance

Mutations in the RTKs as well as dysregulation of its downstream signaling proteins can impair normal DDR. Some RTKs are reported to translocate into the nucleus and their nuclear substrates includes DDR related, RTKs have been implicated in DDR regulation as the canonical RTK downstream proteins have been shown to correlate with DDR regulation (**Figure 2**). RAS constitutive activation and/or mutation are observed frequently in human cancers, and the K-RAS encoding gene is particularly vulnerable to chemical carcinogens [97, 98]. Oncogenic activation of K-RAS leads to an accumulation of replication stress by orchestrating wild-type H- and N-RAS signaling, and triggers the ATR/Chk1 pathways to evade G2 cell-cycle arrest [99]. Oncogenic K-RAS also promotes A-NHEJ by upregulating the expression of DNA ligase III, PARP1, and XRCC1 in leukemia cancer model [100]. The AKT-mediated signaling pathway also regulates DDR. It has been reported that when cells are pretreated with Chk1 inhibitor, inactivation of AKT/PKB pathway can restore radiation-induced Chk1 activation at late G2 cell cycle arrest [101]. Other than Chk1,

AKT is known to inhibit TopBP1 and BRCA1 even though it also positively regulates ATM, ATR, and DNA-PK (reviewed in [102]). In addition to the RAS and AKT pathways, some RTKs can also regulate DDR through other pathways as discussed in the following sections.

Regulation of DDR by the ErbB family

The ErbB family is composed of four receptors, ErbB1 (epidermal growth factor receptor, EGFR), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4). Among them, EGFR and HER2 have been shown to regulate DDR and contribute to therapeutic resistance through both canonical and non-canonical signaling pathways. Using EGFR siRNAs and EGFR small molecule inhibitors, Wei *et al.* demonstrated that EGFR-mediated AKT/ERK pathway upregulates cell cycle regulatory proteins, including cyclin A, B, E, and CDK 1/2, in carcinogenic metal-induced proliferation of triple-negative breast cancer cells (**Figure 3**) [103]. The RAS/MEK/ERK pathway promotes EGFR-mediated radioprotection [104] by affecting gene transcription of the DNA repair proteins. The expression levels of the base repair DNA ligase XRCC1 and the DNA adduct excision protein ERCC1 upregulated under radiation treatment can be attenuated by EGFR inhibitor [105, 106]. By utilizing small molecule inhibitors, radiation-induced and EGFR-mediated XRCC1 upregulation was shown to depend on the RAS/MEK/ERK pathway whereas normal XRCC1 expression is affected by EGFR-mediated PI3K/AKT pathway (**Figure 3**) [107].

Nuclear EGFR also plays an important role in DNA damage repair, including MMR, NHEJ and HR (**Figure 4**). For instance, nuclear EGFR can phosphorylate histone H2B and histone H4. Specifically, EGFR phosphorylates histone H4 at Y-72 to regulate histone H4 methylation [93]. EGF, as well as arsenic, can stimulate nuclear EGFR-mediated phosphorylation and stabilization PCNA via Y211. Phosphorylated PCNA Y211, which has been shown to correlate with poor patient survival, promotes cell proliferation as well as inhibits the endonuclease activity of MutLα, which leads to inhibition of MMR [10, 80, 108]. Yu *et al.* demonstrated that PCNA-derived peptide blocks the EGFR-PCNA complex and suppresses the growth of breast cancer cells [109]. Nuclear EGFR plays a role in HR in many aspects. EGFR phosphorylates ATM at Y370; depletion of EGFR abolishes ATM-mediated foci formation and HR; the ATM-EGFR interaction can be blocked by gefitinib, an EGFR inhibitor [92]. EGFR also interacts with BRCA1 to facilitate HR; the EGFR-BRCA1 interaction as well as BRCA1 nuclear translocation can be blocked by the EGFR inhibitor, lapatinib [110]. These interactions provide molecular basis for the combination therapy of EGFR inhibitor with PARP inhibitor, which induces synthetic lethality in tumor cells, as demonstrated in breast and ovarian cancers [110-112]. Radiation also enhances EGFR nuclear translocation [95, 113]. Nuclear accumulation of EGFR contributes to radio-protection and interferes with DNA repair through interacting and regulating activity of DNAPK [114-117]. Treatment of EGFR monoclonal antibody, cetuximab (C225), promotes the interaction between EGFR, DNAPK, and Ku proteins, which results in a redistribution of DNAPK from the nucleus to cytosol, a critical step in the radiosensitizing role of EGFR blockade [118-120]. EGFR blockade also inhibits cell growth via p27 and maintains cells in G1 phase, which has been shown to also contribute to the radiosensitizing effect of EGFR [121, 122]. In addition to EGFR, HER2 also regulates cell cycle regulation by binding to and colocalizing with cyclin B-bound CDC2 protein. Phosphorylation of CDC2 by HER2 at Y15 then delays entry of cells into M phase and contributes taxol resistance in HER2-overexpressing cancer cells [123]. Inhibition of HER3 also sensitizes cancer cells to radiation therapy by blocking AKT phosphorylation [124], and dual inhibition of EGFR and HER3 can overcome cross-resistance to EGFR inhibition and radiation [125, 126].

MET family regulated DDR

Two RTKs in the MET family, MET (also known as hepatocyte growth factor (HGF) receptor) and Ron (also known as macrophage stimulating 1 (MST1) receptor) also regulate DDR. In lung adenocarcinoma, HGF-induced MET activation inhibits apoptosis through the canonical pathway. Chen *et al.* demonstrated that the $FAK^{-/-}$ mouse embryonic fibroblast cells express higher levels of apoptosis-inducing factor (AIF), which correlates with better therapeutic response to cisplatin treatment. Moreover, AIF expression and cisplatin sensitivity were increased in cells when binding of MET to FAK

was impeded or when MET inactivated [127], suggesting that this FAK-regulated AIF expression is downstream of MET signaling in lung adenocarcinoma cells. MET is also reported to directly phosphorylate PARP1 at Y907 site [128]. Phosphorylated PARP1 is more resistant to the PARP inhibitor, veliparib, and the combination of MET inhibitor and veliparib increased breast cancer cell killing effect. Contrary to MET, Ron phosphorylates PCNA at Y211 through the canonical signaling pathway by activating Ron downstream kinase, c-Abl, an adaptor protein containing SH2 domain [129]. These findings suggested a functional redundancy between Ron receptor and nuclear EGFR on PCNA Y211 regulation.

Future prospect

Although both radio- and chemotherapies have demonstrated significant efficacy in cancer treatment, there are still some patients who have poor response to these treatment methods. Therefore, it is important to address the biological basis of resistance to cancer treatment and to improve the efficacy of radio- or chemotherapies, such as through combination with targeted therapies. In this aspect, there is an urgent and unmet need to develop biomarkers for personalized medicine [130]. As DNA damage adducts and repair capabilities have proven success in predicting cancer risk and therapeutic response, stratifying patients according to the DDR status has emerged as a treatment modality for cancer [45, 131]. While mutations in DDR proteins can serve as predictive markers in cancer treatment [132], DDR proteins are also epigenetically regulated. Thus, the regulatory modifications of DDR proteins as well as mutations and malfunctions of other molecular players involved in DDR are also important factors to consider when stratifying patients for treatment.

Among the potential biomarkers, RTK-related DDR protein phosphorylation is an ideal marker in cancer treatment because RTKs are involved in DDR regulation whereas most of cancer cells develop oncogenic addiction to upregulated RTK signaling pathways. Targeting RTKs, thus, may overcome RTK-mediated therapeutic resistance discussed above. For example, patients with pY211-PCNA and pY370-ATM may benefit from treatments combining EGFR inhibitor with current chemo- or radio- therapies; patients with pY907-PARP1 may benefit from treatment combining MET inhibitor with PARP1 inhibitor. Since oncogenic addiction is important to distinguish the killing effect of RTK inhibitors between normal cells and cancer cells [133], in theory, combining DNA damaging agents with RTK inhibitors can selectively increase genotoxic effects in cancer cells. As the molecular mechanisms underlying RTK-mediated DDR are not yet fully understood, more investigations are needed to further characterize the therapeutic potential of personalized combination therapy targeting this regulatory pathway.

Acknowledgements

We thank Drs. Richard Behringer, Rachel Miller, and Eric Swindell for helpful comments on the manuscript. The work was supported by the following grants: National Institutes of Health (CA109311, CA099031, and CCSG CA016672); National Breast Cancer Foundation, Inc.; Patel Memorial Breast Cancer Endowment Fund; The University of Texas MD Anderson-China Medical University and Hospital Sister Institution Fund; Ministry of Science and Technology, International Research-intensive Centers of Excellence in Taiwan (I-RiCE; MOST 105-2911-I-002-302); Ministry of Health and Welfare, China Medical University Hospital Cancer Research Center of Excellence (MOHW105-TDU-B-212-134003); and Center for Biological Pathways. We apologize to the authors whose original work could not be cited due to space limitation.

Disclosure of conflict of interest

None.

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