Published in final edited form as:

Exp Mol Pathol. 2012 December ; 93(3): 324–333. doi:10.1016/j.yexmp.2012.09.007.

Reversion of the ErbB Malignant Phenotype and the DNA Damage Response

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Abstract

The ErbB or HER family is a group of membrane bound tyrosine kinase receptors that initiate signal transduction cascades, which are critical to a wide range of biological processes. When over-expressed or mutated, members of this kinase family form homomeric or heteromeric kinase assemblies that are involved in certain human malignancies. Targeted therapy evolved from studies showing that monoclonal antibodies to the ectodomain of ErbB2/neu would reverse the malignant phenotype. Unfortunately, tumors develop resistance to targeted therapies even when coupled with genotoxic insults such as radiation.

Radiation treatment predominantly induces double strand DNA breaks, which, if not repaired, are potentially lethal to the cell. Some tumors are resistant to radiation treatment because they effectively repair double strand breaks. We and others have shown that even in the presence of ionizing radiation, active ErbB kinase signaling apparently enhances the repair process, such that transformed cells resist genotoxic signal induced cell death. We review here the current understanding of ErbB signaling and DNA double strand break repair. Some studies have identified a mechanism by which DNA damage is coordinated to assemblies of proteins that associate with SUN domain containing proteins. These assemblies represent a new target for therapy of resistant tumor cells.

Keywords

HER; ErbB; EGFR; DNA repair; Double-strand breaks; ionizing radiation; nuclear compartmentalization; SUN; KASH; LINC

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II. Introduction

II.a. ErbB Signaling and ErbB Targeted Therapies

The ErbB or HER family of tyrosine kinase receptors consists of ErbB1 (the epidermal growth factor receptor (EGFR)/HER1), ErbB2 (p185/neu/ HER2), ErbB3 (HER3), and ErbB4 (HER4) which can form homomeric and heteromeric assemblies (Kokai et al., 1989; Qian et al., 1994). These kinase complexes initiate or participate in a variety of signal transduction cascades, including the MEK and PI-3K pathways. Early studies from this laboratory also showed that disabling the kinase complex with monoclonal antibodies specific for the ectodomain could reverse aspects of the malignant phenotype (Drebin et al., 1985; Drebin et al., 1986). The use of two monoclonal antibodies reactive with distinct regions of the ectodomain enhances the reversal of phenotype *in vitro* and completely eradicates some tumors *in vivo* in animal models (Drebin et al., 1988). These approaches were advanced to the clinic and single and dual antibody therapy are now applied to human diseases such as ErbB2 driven breast and stomach cancer (Baselga et al., 2010; Cortes et al., 2012; Portera et al., 2008; Smyth and Cunningham, 2012).

While a great deal of effort has been devoted to the development and implementation of targeted therapy aimed at disabling ErbB signaling with monoclonal antibodies and small molecule tyrosine kinase inhibitors, cells frequently become resistant to these treatments even when combined with genotoxic injury such as chemotherapy or radiation therapy. ErbB2/neu and EGFR transformed cells, as mentioned are inherently resistant to radiation-induced apoptosis.

The ErbB family of receptors are frequently over-expressed or activated in a variety of cancers. ErbB2 is amplified in approximately 25% of breast cancer patients and amplification is associated with poor prognosis and decreased survival (Riemsma et al., 2012). In addition, EGFR is over-expressed or mutated in a variety of cancers including non-small cell lung cancer and head and neck squamous cell carcinomas (Foley et al., 2012; Taylor et al., 2012). Activation of the ErbB kinase induces a variety of effects on the cell including increased proliferation, migration, survival, evasion of apoptosis, metastasis, and resistance to chemo- and radiotherapeutics.

II.b. Radiation and DNA double strand break

In response to ionizing radiation (IR), several modifications of DNA occur including the ionization of bases and sugars, the formation of DNA-DNA and DNA-protein crosslinks, and single and double strand breaks (SSB and DSBs, respectively). In fact, exposure to 1Gy of radiation causes 16 to 40 DSBs in a diploid genome (Barker and Powell, 2010). When ionizing particles pass through water, they generate free radicals through the radiation-induced lysis of water molecules (Magnander and Elmroth, 2012). Hydroxyl free radicals then interact with DNA, which can lead to a SSB through the removal of a hydrogen atom from the deoxyribose (Masuda and Kamiya, 2012). Two relatively close SSBs on opposite strands of DNA or the presence of a SSB during DNA replication would produce a DSB (Rodriguez-Rocha et al., 2011). At the molecular level, oxidation damage from metabolically generated free radicals or IR are identical; however, IR is much more harmful due to the production of clustered DNA damage sites (two or more lesions formed within one or two helical turns) (Eccles et al., 2011).

The DNA DSB is a dominant form of damage caused by IR (Huang et al., 1996). DSBs also occur in response to endogenous signals such as those mediated by reactive oxygen species generated during metabolic processes as well as during antibody generation through V(D)J recombination (Mahaney et al., 2009). Cells encounter several DSB per day (Burma et al.,

2006) and failure to efficiently repair these breaks can lead to disastrous outcomes including cell death or genomic instability.

Cells can repair the DSB lesion using either homologous recombination (HR) repair or non-homologous end-joining (NHEJ) repair. NHEJ is frequently used in mammalian cells. HR, which is dependent on BRCA1 functions and requires a sister chromatid, occurs most frequently in late S or G2. Conversely, NHEJ can take place at any stage of the cell cycle and involves the joining together of two ends (Mahaney et al., 2009). Deficiency in BRCA1 limits HR functionality and triggers the more error-prone NHEJ. The NHEJ process has the potential to be a transforming event if joining limits a tumor suppressor gene or activates an oncogene. However, since the majority of the higher eukaryotic genome is noncoding the likelihood of transforming events is low.

II.c. ErbB and DSB repair

Activation of ErbB1 with either a ligand or a constitutively active mutant form enhances the efficiency of the DSB repair pathway (Kriegs et al., 2010; Mukherjee et al., 2009). However disabling ErbB prior to radiation renders the cells more sensitive to the genotoxic-induced death seen with normal non-transformed cells (O'Rourke et al., 1998a). Combinations of ErbB targeted therapy to reverse phenotype followed by IR are now used for treatment of certain human tumors.

III. ErbB Signaling

The ErbB family of receptors consists of ErbB1 (the epidermal growth factor receptor (EGFR)/HER1), ErbB2 (p185/neu/ HER2), ErbB3 (HER3), and ErbB4 (HER4). These receptor tyrosine kinases (RTKs) are single pass membrane glycoproteins whose intracellular C-terminal domain contains the tyrosine kinase domain and N-terminal extracellular domain contains the ligand-binding and dimerization domains. The extracellular portion of the receptor is composed of four domains, known as Domains I, II, III, IV (the role of these domains in receptor activation is discussed below). Known ligands for EGFR include epidermal growth factor (EGF), transforming growth factor- α (TGF- α), heparin-binding EGF (HB-EGF), amphiregulin (ARG), epiregulin, and betacellulin. HER3 and HER4 are activated by the four neuregulins (NRG1-4) and, in addition, HER4 is also activated by HB-EGF, betacellulin, and epiregulin (for more in-depth information, please see (Fuller et al., 2008)).

Activation of the ErbB family members differs from other RTKs. EGFR is often considered to be the model receptor of the family; therefore, activation of the receptors will be discussed in the context of EGFR. In contrast to other RTKs, which are activated in the presence of a bivalent ligand that induces dimerization by interacting with two receptors (in a 1:2 ratio of ligand to receptor), one EGF molecule interacts with a single EGF receptor; thus, an activated receptor is constituted by a 2:2 ratio of ligand to receptor (Garrett et al., 2002; Ogiso et al., 2002). Briefly, in the absence of a ligand, EGFR is maintained in an auto-inhibitory state in which the dimerization arm within Domain II is buried in Domain IV. Following ligand binding to Domain I, the receptor undergoes a conformational change whereby Domains I and II rotate exposing the dimerization arm, thus allowing homo- or heterodimerization and subsequent receptor activation (the auto-inhibitory interaction between Domains II and IV in the absence of the ligand functions to ensure sufficient separation of Domains I and III, thereby preventing aberrant activation of the receptor) (Ferguson et al., 2003). The fully activated receptor is described as having EGF bound to Domains I and III (which has been demonstrated by crystallographic and biochemical studies (Garrett et al., 2002; Kohda et al., 1993; Lax et al., 1988; Ogiso et al., 2002)) and Domain II in a position such that the dimerization arm is positioned for dimerization

(Ferguson et al., 2003). It is important to point out that ErbB2 does not have an identified ligand and the receptor appears to be maintained in a constitutively active state with the dimerization arm always accessible, thus enabling heterodimerization with a ligand-bound, activator partner at any point. ErbB2 is also the preferred heterodimerization partner for the other ErbB receptors (Graus-Porta et al., 1997; Graus-Porta et al., 1995) and the combination of ErbB2/ErbB3 leads to the greatest transformation potential (Wallasch et al., 1995).

Dimerization induces phosphorylation of tyrosine residues on the intracellular portion of the receptors, which activates downstream signaling through recruitment of intracellular adaptor proteins, kinases, and other signal transducing proteins. ErbB activated pathways include the phospholipase C- γ /protein kinase C, Ras/Raf/MEK/ERK, PI-3K/Akt, and JAK/STAT; however, in relation to the topic at hand, only the ERK1/2 and PI-3K/Akt will be discussed further. The receptor signal is dampened by internalization, which is mediated through receptor clustering in clathrin-coated pits. Upon internalization, the receptor can either be recycled back to the membrane or it can be degraded in the lysosomes. EGF-bound EGFR is stable as it traffics through the endosomes; however, in the lysosome, the EGF-EGFR interaction is dissociated (Roepstorff et al., 2008). Ligand shedding from the receptor in the lysosome is consistent with the data demonstrating pH sensitivity of the EGF-EGFR interaction (Ferguson et al., 2003). Clearly, the pathway of receptor activation, signaling, and degradation is quite complex.

IV. DNA Repair Mechanisms

IV.a. Double Strand Break Repair

The DSB repair pathway is a complex process that requires several proteins. Certain known steps involved in DSB repair include: 1) recognition of the lesion, mediated by the DNA protein kinase (DNA-PK) holoenzyme; 2) end processing, achieved by Artemis and the DNA polymerase family X (composed of the terminal deoxyribonucleotidyltransferase (TdT) and the polymerases γ and μ and 3) ligation of the two ends, accomplished by the X-ray-complementing Chinese hamster gene 4 (XRCC4)/DNA Ligase IV (XL) complex as well as the XRCC4-like factor, XLF, also known as Cernunnos. In addition to mediating DSB repair in response to IR, DNA-PK is also a critical element to the V(D)J recombination event required for the diversity of antibodies. Studies have shown that nonsense mutations of DNA-PKcs in mice, dogs, and horses produces a SCID phenotype that is attributed to deficiency in DNA-PKcs-mediated V(D)J rearrangement (Blunt et al., 1996; Ding et al., 2002; Shin et al., 1997).

It is important to point out that in response to DNA damage, irradiation-induced foci (IRIF) formation is also a key process. Histone H2A.X is a crucial determinant of the IRIF formation and DNA damage response because genetic ablation of H2A.X increases radiosensitivity in vitro and in vivo (Bassing et al., 2002; Celeste et al., 2002). In response to DNA double strand breaks, histone H2A.X is phosphorylated on Ser 139, which is otherwise known as γ -H2AX (Rogakou et al., 1998). Subsequent studies found that ATM, ATR, and DNA-PK can phosphorylate H2A.X (Burma et al., 2001; Ward and Chen, 2001). H2A.X Tyr 142 is basally phosphorylated; however, following DNA damage under conditions of repair and survival, Tyr 142 is dephosphorylated dependent on EYA (Cook et al., 2009). Further, MDC1 (mediator of DNA damage checkpoint protein 1) was identified as a γ -H2AX interacting protein and the interaction between these proteins is essential for the formation of IRIF foci and radioresistance (Stucki et al., 2005). Moreover, a recent study found that MCPH1 is able to bind a di- γ -H2A.X, which is phosphorylated on Tyr 142 as well as Ser 139 (Singh et al., 2012). Importantly, the study demonstrated that inhibition of Tyr 142 phosphorylation through mutation to phenylalanine prematurely caused

dephosphorylation of γ -H2A.X, thus impairing γ -H2A.X kinetics and potentially disrupting the DDR (Singh et al., 2012).

IV.a.i. The Ku Heterodimer—The Ku 70 and 80kDa heterodimeric complex recognizes DSBs where its ring-like structure, which does not appear to discriminate between nucleotides, binds free ends of DNA with high affinity (Walker et al., 2001). Ku is the first protein to accumulate at sites of damage following IR (Kim et al., 2005; Mari et al., 2006). Following binding to the free ends of damaged DNA, Ku proteins undergo a conformational change (Lehman et al., 2008) and translocate inward by approximately one helical turn, in an ATP-independent manner (Yoo and Dynan, 1999). Upon recruitment of the catalytic subunit of DNA-PK (DNA-PKcs) to the lesion, formation of the DNA-PK holoenzyme is complete. The Ku complex is also essential for the recruitment of XRCC4 (Mari et al., 2006) and XLF (Yano and Chen, 2008) to sites of DSB. Therefore, the Ku complex not only represents the point of initiation of DSB repair pathway, but also plays a pivotal role in the assembly of the machinery to repair the lesions.

IV.a.ii. The DNA-dependent Protein Kinase Catalytic Subunit—Formation of the active DNA-PK holoenzyme requires the interaction between Ku and DNA-PKcs as well as double stranded DNA (Gottlieb and Jackson, 1993; Suwa et al., 1994). Ku interacts with DNA-PKcs through its C-terminus (Gell and Jackson, 1999; Singleton et al., 1999), which is essential for IR-resistance (Falck et al., 2005). Also, DNA-PKcs interacts with Ku through its C-terminus (Jin et al., 1997). A leucine-rich DNA binding domain mediates the interaction between DNA-PKcs and DNA (Gupta and Meek, 2005). The C-terminal kinase domain of DNA-PKcs is situated above the N-terminal ring that forms a predominantly helical “concave, cradle like structure” (Sibanda et al., 2010). Cells lacking DNA-PKcs are unable to repair DSBs and are sensitive to IR (Khanna and Jackson, 2001; Lees-Miller et al., 1995), leading to the supposition that DNA-PKcs is a critical component to the DSB repair process.

The principal phosphorylation target of DNA-PKcs is itself. After DSB recognition by Ku and assembly of DNA-PK, the two holoenzymes form a synapse leading to DNA-PKcs autophosphorylation (Weterings et al., 2003). DNA-PKcs kinase inactive mutants fail to perform the DSB repair process in cells lacking DNA-PKcs (Kurimasa et al., 1999) and inhibition of DNA-PKcs impedes repair (Baumann and West, 1998); thus, DNA-PK kinase activity is essential for DSB repair. DNA-PKcs autophosphorylation inhibits further kinase activity and dissociates DNA-PKcs from Ku (Chan and Lees-Miller, 1996; Merkle et al., 2002), which is necessary for end-processing events (Calsou et al., 1999; Weterings et al., 2003). Two autophosphorylation clusters, referred to as ABCDE (Thr 2609, 2620, 2638, and 2647 as well as Ser 2612, 2624 and 3205) and PQR (Ser 2023, 2029, 2041, 2053, and 2056), which control end processing have been described. Mutation of individual phosphorylation residues in either ABCDE or PQR clusters does not affect radiosensitivity; however, combinatorial mutation of residues to alanines increases radiosensitivity with more pronounced effects in the ABCDE cluster (Block et al., 2004; Cui et al., 2005; Ding et al., 2003; Douglas et al., 2002; Meek et al., 2007). Furthermore, ABCDE *trans*-phosphorylation and PQR dephosphorylation promotes access to ends which favors end processing and likely recruits the XL complex (Cui et al., 2005; Meek et al., 2007; Reddy et al., 2004). Further analysis of individual residues showed that in response to IR, DNA-PKcs Thr 2609 is phosphorylated and localizes at DNA damage foci (Chan et al., 2002) and mutation of Thr 2638 and 2647 to alanines also radiosensitizes cells but does not affect NHEJ activity (Soubeyrand et al., 2003). The ABCDE and PQR clusters contribute to end processing but do not appear to affect end-joining activity.

IV.a.iii. End Processing—Because DSBs are complex and variable in nature, end processing requires several different types of enzymes. Specifically, DNA ends may require the removal of blocking groups, exonuclease processing, or filling in gaps in order for proper ligation to take place.

Artemis is a nuclease that exhibits both 5'-3' exonuclease and endonuclease activity (the presence of DNA-PK favors endonuclease activity) as well as the ability to resolve hairpin structures (Ma et al., 2002; Ma et al., 2005b). The N-terminus of Artemis contains a β -lactamase/ β -CASP nuclease domain and the C-terminus is a phosphorylation target. Artemis expression sufficiently complements the V(D)J recombination defect observed in radiosensitive-SCID fibroblasts (Moshous et al., 2001). MEFs from Artemis null mice are radiosensitive (Rooney et al., 2002); however, immune-depletion of Artemis in HeLa cell lysates suggests that it is dispensable for DSB repair (Zhang et al., 2004). In addition to its nuclease activity, Artemis can remove phosphoglycolate-blocking groups (Povirk et al., 2007), a necessary event prior to ligation. Although Artemis is basally phosphorylated and phosphorylated Artemis is detected at sites of damage, no function of this phosphorylation event has been reported (Chen et al., 2005; Goodarzi et al., 2006; Ma et al., 2005a; Poinsignon et al., 2004; Riballo et al., 2004; Soubeyrand et al., 2006; Zhang et al., 2004). Conversely, Artemis recruitment to sites of DNA damage requires DNA-PKcs kinase activity (Drouet et al., 2006; Ma et al., 2002) and autophosphorylation of DNA-PKcs is necessary for Artemis activity (Goodarzi et al., 2006; Weterings et al., 2009). Therefore, DNA-PKcs autophosphorylation may provide Artemis access to DNA, but phosphorylation of Artemis does not appear to be a requirement.

In certain instances, prior to ligation, gap filling is required. Members of the DNA polymerase X family, DNA polymerases μ and λ as well as TdT are implicated in DSB repair. Support for this concept is derived from the findings that polymerase μ content increases following DNA damage and accumulates at damage foci as well as complexes with Ku and the XL complex (Mahajan et al., 2002). Perhaps the specific polymerase that is used may depend on the nature of the lesion. Polymerase λ is template dependent (Garcia-Diaz et al., 2002) while polymerase μ is either template dependent or independent, but exhibits peak activity in the presence of a template (Dominguez et al., 2000; Gu et al., 2007a). Interestingly, the lymphocyte-specific TdT does not require a template (Moon et al., 2007). Surprisingly, exposure of MEFs lacking polymerases μ and γ to radiation demonstrated a lack of sensitivity (Bertocci et al., 2006), implying that these polymerases are not necessary for general DSB repair and may only be required for specific types of lesions.

IV.a.iv. Ligation—The two ligation-competent DNA ends are joined together by the XL complex. Formation of this highly stable, direct interaction between XRCC4 and DNA Ligase IV stimulates ligase activity and is necessary for NHEJ as well as V(D)J recombination (Critchlow et al., 1997; Grawunder et al., 1997; Grawunder et al., 1998; Li et al., 1995). The XRCC4 protein is composed of a globular head domain at the N-terminus and a helical stalk at the C-terminus. Two XRCC4 dimers ultimately form a tetramer that takes on a “barbell-like” appearance (Junop et al., 2000). In addition to DNA Ligase IV, XRCC4 interacts with other DSB repair proteins as well as DNA (Ahnesorg et al., 2006; Costantini et al., 2007; Modesti et al., 1999; Nick McElhinny et al., 2000). Ku recruits the XL complex to DNA and the Ku:XL complex together promotes efficient ligation activity (Nick McElhinny et al., 2000). While Ku, but not DNA-PKcs, is required for the recruitment of XRCC4 to damage sites (Mari et al., 2006), the presence of DNA-PKcs enhances its interaction with DNA Ligase IV, which is stabilized in the presence of wortmannin (Costantini et al., 2007).

Both XRCC4 and DNA Ligase IV are essential components to the DSB repair process. Cells lacking XRCC4 are radiosensitive (Li et al., 1995) whereas genetic ablation of the DNA Ligase IV gene in mice results in embryonic lethality (Barnes et al., 1998; Frank et al., 1998); however, MEFs from DNA Ligase IV null mice are severely radiosensitive (Frank et al., 1998). Importantly, these studies are supported by analysis of cell lines derived from humans with radiosensitivity or immune dysfunction (O'Driscoll et al., 2001; Riballo et al., 1999).

XLF is a recently described XRCC4-interacting protein, which, similar to XRCC4, is crucial to radioresistance and DSB repair and specifically stimulates DNA Ligase IV activity (Ahnesorg et al., 2006; Hentges et al., 2006). Structurally, XLF is similar to XRCC4 with a few differences. Notably, the stalk is larger in XRCC4 and the head and stalk interact differently, thus the angle at which the helical stalk protrudes from the globular head differs between the proteins (Andres et al., 2007; Li et al., 2008). XLF DNA binding is dependent on the length of the DNA molecule (Lu et al., 2007). Intriguingly, XLF stimulates the ligation of incompatible DNA ends (Gu et al., 2007b; Tsai et al., 2007).

In all, these studies put forth a model whereby following detection of a DSB and assembly of DNA-PK, synapse formation induces DNA-PKcs autophosphorylation on multiple residues that will ultimately cause DNA-PKcs to dissociate from the DSB; however, partial phosphorylation is necessary to maintain DNA-PKcs at the lesion so as to recruit to the XL complex, and then subsequent autophosphorylation on other residues would lead to the dissociation of the repair machinery.

IV.b. Mismatch repair

Although DNA mismatch is unlikely a major consequence of IR and DNA mismatch repair is not usually involved after DSB, mismatch repair proteins such as MSH2 are reported to be involved in the cellular response to radiation in mammalian cells (Martin et al., 2010). Using MSH2-null cells, which have inefficient G2/M checkpoint after x-ray induced chromosomal damage, Franchitto and colleagues have revealed a role for MSH2 to facilitate the relocation of MRE11 and RAD51 to damaged sites (Franchitto et al., 2003). We reported the association of BRCA1 and MSH2 (Wang et al., 2001). The BRCA1 DNA repair complex can dynamically include additional repair proteins (e.g. MSH6, MLH1, ATM, BLM, RAD50, MRE11, NBS1) and play different roles ranging from transcription-coupled repair to repairs related to chemical or radiation induced abnormal DNA structures (Wang et al., 2000).

IV.c. Therapies Targeting DNA Repair

The roles of BRCA1 in both single and double strand repair explain its function as a general tumor suppressor to protect genomic integrity in normal cells (Wang et al., 1998). However, tumor cells also rely on BRCA1 and its functionally related protein BRCA2 to overcome DNA damages induced by chemo- or radiotherapeutic agents (Murray et al., 2007). This provides opportunity for the development of treatment for some types of tumors that are deficient in BRCA1 and BRCA2 (Aly and Ganesan, 2011). For example, DNA repair, especially the SSB, requires some chromatin changes that are controlled by poly(ADP-ribose) polymerase (PARP). Inhibition of PARP leads to accumulation of SSB damages and subsequently DSB DNA damage during DNA replication in S phase. BRCA1 and BRCA2 are enlisted at this point to rescue the DNA repair and cell cycle progression for tumor cells. Without functional BRCA1/2, tumor cells will not be able to survive the DNA damages. For this reason, it is not unexpected to see clinical response of BRCA1/2 deficient breast cancer (Tutt et al., 2010) or ovarian cancer patients (Audeh et al., 2010) to PARP inhibitor treatment.

V. ErbB Signaling and DNA Repair

IR activates the ErbB family signaling cascades and the effector kinases are intimately involved in mediating IR resistance. Constitutive EGFR activation leads to radioresistance, a phenotype that is lost in DNA-PKcs (–/–) MEFs and a DNA-PKcs inhibitor blocks the constitutively active EGFR-mediated radioresistance (Mukherjee et al., 2009). Indeed multiple studies have analyzed this subject area in-depth from worms (Weidhaas et al., 2006) to mice (see below) and this section will attempt to summarize the major findings.

V.a. Involvement of the Epidermal Growth Factor Receptor

EGFR is activated following exposure to IR and inhibition of EGFR by various means blocks IR-induced EGFR stimulation and downstream signal transduction. EGFR-enhanced DSB repair is mediated primarily through NHEJ (Golding et al., 2009), implying a role for DNA-PK. Indeed, use of the constitutively active EGFRvIII (the principal EGFR mutation found in glioma) demonstrated that DNA-PKcs is downstream of activated EGFR (Mukherjee et al., 2009). Dominant negative blockade of EGFR inhibits both IR- and EGF-induced EGFR phosphorylation and, in response to repeated dosages of IR, impedes proliferation of mammary carcinoma cells in culture (Contessa et al., 1999) as well as conferring radiosensitivity to xenograft tumors in mice as measured using an ex vivo colony formation assay (Lammering et al., 2001). Use of a monoclonal antibody to EGFR (Huang et al., 1999) increases radiosensitivity in head and neck squamous cell carcinoma (SCC) cell lines. Furthermore, gefitinib treatment radiosensitizes cell lines representing SCC of the head and neck as well as oral cancer, non-small cell lung carcinoma (NSCLC), epidermoid carcinoma of the vulva, and glioblastoma (Huang et al., 2002; Shintani et al., 2003; Solomon et al., 2003; Stea et al., 2003; Tanaka et al., 2008) and alters angiogenic properties such as vascular endothelial growth factor production and blood vessel infiltration (Huang et al., 2002; Shintani et al., 2003; Solomon et al., 2003). In addition, erlotinib treatment blocks IR-induced EGFR activation in NSCLC and head and neck SCC cell lines in vitro as well as reduces tumor volume in vivo (Chinnaiyan et al., 2005). In good agreement, depletion of EGFR with RNAi radiosensitizes glioma cells in vitro and in vivo (Cui et al., 2005). Inhibition of EGFR reduces radiation-induced migration of head and neck SCC cells (Pickhard et al., 2011), which supports the association of radioresistance with epithelial-to-mesenchymal transition (Holz et al., 2011; Skvortsova et al., 2010). Finally, a recent study reported that inhibition of EGFR in the presence of IR results in senescence, rather than apoptosis (Wang et al., 2011), suggesting that a more thorough examination may be warranted.

V.b. Involvement of ErbB2

In addition to the EGFR, ErbB2 is also critical to mediating the DNA DSB response following IR exposure. Disabling ErbB2 with the dominant negative ErbB2-T691stop, which is exclusively the ectodomain of p185 that binds to EGFR and stops downstream signaling (O'Rourke et al., 1998b; O'Rourke et al., 1997), causes a radiosensitive phenotype (O'Rourke et al., 1998a). Monoclonal antibody blockade of ErbB2 radiosensitizes cells and inhibits DNA repair as well as reduces tumor volume when combined with irradiation in mice (Pietras et al., 1999). Similar findings were reported in cell lines with varying degrees of ErbB2 expression when treated with trastuzumab (Liang et al., 2003). Congruently, Park and colleagues showed that use of a peptide with functional conservation to the p185^{HER2/neu} antibody results in IR-induced apoptosis in astrocytoma cells (Park et al., 2000). Similarly, silencing ErbB2 with siRNA in an ErbB2 overexpressing breast adenocarcinoma cell line (SK-BR-3) results in radiosensitivity (No et al., 2009). The heterodimeric pairing of ErbB2 with EGFR is important in mediating signal transduction in response to irradiation; however, this is not the case for EGF-stimulated signaling (Toulany

et al., 2010). As expected, treatment of breast cancer cells that overexpress EGFR with lapatinib (dual EGFR/HER2 kinase inhibitor) radiosensitizes the cells (Zhou et al., 2004). Clearly, the ErbB family of receptors is critical in mediating the cellular response to IR.

In addition, p185^{HER2/neu} is also involved in the repair of chemotherapeutic agent-induced DNA damage, but the mechanism is not clearly understood. Treatment with the antibody to p185^{HER2/neu} delays the repair of interstrand crosslinks, but not intrastrand or DNA strand breaks, caused by cisplatin (Boone et al., 2009). We have reported an inverse correlation between p185^{HER2/neu} activity and BRCA1 tyrosine phosphorylation (Zhang et al., 1997), a post-translational modification that is dependent on ATM and c-Abl activation and induced by radiation (Foray et al., 2002).

V.c. Signaling Downstream of the Receptor: A Potential Feedback Mechanism

Following the activation of ErbB family receptors, signal transduction pathways, including the PI-3K/Akt and MEK pathways, are activated. There appears to be more than one pathway responsible for activating the DSB repair machinery. Constitutive Ras activation leads to the secretion of the EGFR ligands ARG and TGF- α (ARG was the most potent), which activate EGFR and therefore increase radioresistance (Minjgee et al., 2011). Inhibition of the Akt pathway in constitutively active K-Ras cells dramatically inhibits repair of DNA DSBs following radiation in comparison to wild-type K-Ras expressing cells, which do not repair DSBs as efficiently in the face of irradiation (Toulany et al., 2006). Importantly, radiation-induced secretion of TGF- α is conserved from cell culture to xenograft models to humans undergoing radiation therapy for hormone refractory prostate cancer (Hagan et al., 2004). These reports would suggest that in response to radiation, a feedback loop exists whereby activation of the Ras pathway mediates production of paracrine and/or autocrine factor(s), which subsequently stimulate the PI-3K/Akt pathway.

V.d. Is PI-3K/Akt the Direct Mediator of ErbB-activated DSB Repair Response?

Several studies imply that the Akt pathway is directly involved in the DSB repair process. Inhibition or silencing of Akt prior to exposure of lung adenocarcinoma cell lines to IR results in an impaired ability to mediate DNA DSB repair (Toulany et al., 2008). Expression of a myristylated, constitutively active Akt accelerates DNA repair kinetics following IR treatment (Mukherjee et al., 2009). In addition, Akt is a substrate for DNA-PK (Park et al., 2009) and several reports have shown Akt to interact with DNA-PKcs even in the absence of Ku80 (Bozulic et al., 2008; Toulany et al., 2008; Toulany et al., 2012). Overexpression of NSB1, a critical protein in DSB repair, activates Akt (Park et al., 2009). Perhaps the most direct evidence supporting a dominant role of the Akt pathway relates to studies of Bozulic and colleagues. In their study, the authors found that activated Akt (pSer 473) is present at foci following irradiation, which is dependent on the phosphoinositide-dependent kinase 1 and DNA-PKcs (Bozulic et al., 2008). Additionally, Akt is required for the radiation-dependent increase of p21, the reduction of which leads to decreased survival following IR (Kokunai et al., 2001), and p21 expression is sufficient to reduce the observed apoptosis in Akt null cells following exposure to radiation (Bozulic et al., 2008). Finally, Akt is needed, at least in part, for DNA-PKcs to accumulate at DNA DSB foci as well as DNA-PKcs kinase activity (Toulany et al., 2012).

The relationship between Akt and DSB repair is critical to stem-cell resistance to radiation. In cell culture, brain tumor stem cells are dependent on EGFR for proliferation (Griffero et al., 2009) and sphere formation (Soeda et al., 2008). Based on these observations, Kang and colleagues found that brain tumor stem cells are radioresistant and, specifically in stem-like cells, following IR exposure, gefitinib treatment reverses EGFR and Akt phosphorylation (Tyr 1068 and Ser 473, respectively) as well as decreases DSB repair and increases

radiosensitivity (Kang et al., 2012). In response to IR, cancer stem cells from the perivascular niche of medulloblastoma activate the PI-3K/Akt pathway and enter into p53-dependent cell cycle arrest; however, inhibition of Akt in the presence of IR results in apoptosis (Hambardzumyan et al., 2008).

V.e. Clinical Trials Combining Radiotherapy with Disabling ErbB Kinase Activity

Several clinical trials examining the combination of radiotherapy (RT) with disabling ErbB kinase activity have been undertaken. Perhaps the most successful example is a Phase III trial of patients with SCC of the head and neck, which showed an overall survival of 49 months for patients receiving cetuximab combined with RT compared with 29.3 months for patients receiving RT alone (Bonner et al., 2006). In the follow up study, the authors reported a higher five-year survival for patients in the cetuximab plus RT arm (45.6%) compared to those in the RT alone arm (36.4%) (Bonner et al., 2010). In good agreement, two other studies reported similar findings (Curran et al., 2007; Heron et al., 2011). Surprisingly, two studies testing the possibility of gefitinib in combination with RT for SCC of the head and neck found that although the treatment regimen was well tolerated, there is no clinical benefit (Caponigro et al., 2008; Gregoire et al., 2011).

Combinations of RT with cetuximab, trastuzumab, and gefitinib are being explored in other cancers as well. Several phase II studies examining cetuximab plus RT for patients with NSCLC suggest a potential for use of this regimen, but further studies are necessary before making conclusions (Blumenschein et al., 2011; Jatoi et al., 2010; Jensen et al., 2011). Additionally, a phase II/III study is currently underway to determine the suitability of using cetuximab with chemoradiotherapy in patients with carcinoma of the esophagus (Hurt et al., 2011). The use of trastuzumab combined with RT as a treatment for breast cancer was evaluated in a phase II trial and the treatment regimen was well tolerated (Horton et al., 2010). Finally, a phase I/II trial of gefitinib and RT treatment of non-metastatic prostate cancer revealed that the combination is relatively well tolerated and suggests that further exploration may be warranted (Joensuu et al., 2010).

VI. Role of the Nuclear Envelope in DNA Damage Repair

The nuclear envelope is composed of an outer nuclear membrane (ONM), which is continuous with the endoplasmic reticulum and an inner nuclear membrane (INM) associated with the nuclear lamin network. In between the two membranes is the perinuclear space (PNS). The cytoskeleton is connected to the nuclear lamina by the LINC (linker of nucleoskeleton and cytoskeleton) complex, which consists of the SUN (Sad1-UNC-84 homology) and KASH (Klarsicht, ANC-1, and Syne homology) domain containing nuclear envelope proteins. UNC-84 requires lamin to integrate into the INM (Lee et al., 2002). The KASH domain containing protein ANC-1 is dependent on UNC-84 to anchor in the ONM and is crucial to linking the cytoskeleton to the nucleus (Starr and Han, 2002). We and others have recently resolved the crystal structures of the evolutionarily conserved core of the LINC complex (Figure 1) and revealed at atomic level the correlation between the assembly of the LINC complex and cell migration (Sosa et al., 2012; Wang et al., 2012; Zhou et al., 2012). These studies strongly indicate that the C-terminal SUN domain forms a homo-trimer, which functions as a primary KASH-binding unit. Since KASH binds to the homo-trimeric interfaces of the SUN domain, mutations disrupting homo-trimerization of SUN domain disable assembly and cellular functions of the LINC complex. More importantly, our studies support a regulated networking model of the LINC complex in PNS, wherein the coiled coil motifs of the SUN proteins may differentially modulate KASH binding and therefore control the SUN-KASH-mediated force (de)coupling between the nuclear periphery and the cytoskeleton (Wang et al., 2012). It is worth noting that

mechanical forces transduced by LINC complexes across the nuclear envelope affect a variety of cellular processes especially those happening at the nuclear periphery.

Recently, two studies in yeast show that the SUN domain containing nuclear envelope protein Mps3 is involved in maintaining genomic integrity during the process of DSB repair. Utilizing chromosome conformation capture analysis, Oza and colleagues found that the region of DSB becomes sequestered in the nuclear periphery and thus has reduced interaction with the intact genome (Oza et al., 2009). This observation is consistent with a previous report showing that DSBs can be translocated and restricted to the nuclear periphery (Nagai et al., 2008). Intriguingly, the peripheral localization of persistent DSBs is dependent on the N-terminus of Mps3 (Oza et al., 2009). In addition, the N-terminus of Mps3 was also shown to be critical in localizing telomeres to the nuclear periphery (Bupp et al., 2007; Schober et al., 2009), thus preventing telomeres from becoming potential recombination partners. Hence, Mps3 is a critical participant in the process of restriction of both DSBs and telomeres in a sub-compartment where potential errors during repair may be prevented. Although such mechanisms have not been reported for mammalian systems, a recent study shows that loss of SUN1 and SUN2 proteins may lead to changes in the DNA damage response and perhaps increased levels of genomic instability (Lei et al., 2012).

Mps3 is also critical to spindle pole body (centrosome equivalent in yeast) assembly as well as telomere anchoring (Antoniacci et al., 2007; Bupp et al., 2007; Jaspersen et al., 2002; Jaspersen et al., 2006; Nishikawa et al., 2003). It is important to point out that the mammalian counterparts of Mps3, including SUN1 and SUN2, play a role in maintaining appropriate coupling between the centrosome and the nucleus (Zhang et al., 2009).

VII. Future Perspective

The subject of DSB repair is quite complex and requires further investigation. Based on the convincing evidence that yeast deal with persist DSBs by compartmentalizing them to the nuclear periphery and Mps3 is essential to this process, it is tempting to speculate a conserved mechanism in mammalian cells. The SUN1 and/or SUN2 proteins could be the mediators of the mammalian nuclear compartmentalization process (modeled in Figure 2). In addition, it is now well accepted that members of the ErbB family are present in the nucleus and can function as transcriptional regulators and likely would influence repair machinery (Dittmann et al., 2010). Therefore, the possibility exists that ErbB family members could act on SUN proteins and this interplay may modulate the kinetics or efficiency of repair.

Abbreviations Used

ARG	amphiregulin
ATM	ataxia telangiectasia mutated
ATR	ATM and Rad3-related
BRCA1/2	breast cancer type 1/2 susceptibility protein
DNA-PK	DNA dependent protein kinase
DSB	double strand break
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ERK	extracellular-regulated kinase

H2A.X	Histone H2A variant X
HB-EGF	heparin-binding EGF
HER	human epidermal growth factor receptor
HR	homologous recombination
INM	inner nuclear membrane
IR	ionizing radiation
IRIF	irradiation-induced foci
JAK/STAT	janus kinase/signal transducer and activator of transcription
KASH	klarsicht, anc-1, and syne homology
LINC	linker of nucleoskeleton and cytoskeleton
MCPH1	microcephalin
MDC1	mediator of DNA damage checkpoint protein 1
MEFs	mouse embryonic fibroblasts
MEK	mitogen-activated protein kinase kinase
MRE11	meiotic recombination 11
MSH	MutS homolog
NHEJ	non-homologous end-joining
NRG	neuregulin
NSCLC	non-small cell lung carcinoma
ONM	outer nuclear membrane
PARP	poly(ADP) ribose polymerase
PI-3K	phosphoinositide 3-kinase
PNS	perinuclear space
RT	radiation therapy
RTK	receptor tyrosine kinase
SCC	squamous cell carcinoma
SCID	severe combined immunodeficiency
SSB	single strand break
SUN	sad1-unc-84 homology
TdT	terminal deoxyribonucleotidyltransferase
TGF-α	transforming growth factor-alpha
V(D)J	variable, diverse, and joining
XL complex	XRCC4/DNA Ligase IV complex
XLF	XRCC4-like factor
XRCC4	X-ray-complementing Chinese hamster gene 4
γ-H2AX	Histone H2A.X phosphorylated on Ser 139

References

- Ahnesorg P, et al. XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell*. 2006; 124:301–313. [PubMed: 16439205]
- Aly A, Ganesan S. BRCA1, PARP, and 53BP1: conditional synthetic lethality and synthetic viability. *Journal of molecular cell biology*. 2011; 3:66–74. [PubMed: 21278454]
- Andres SN, et al. Crystal structure of human XLF: a twist in nonhomologous DNA end-joining. *Molecular cell*. 2007; 28:1093–1101. [PubMed: 18158905]
- Antoniacci LM, et al. The nuclear envelope and spindle pole body-associated Mps3 protein bind telomere regulators and function in telomere clustering. *Cell cycle*. 2007; 6:75–79. [PubMed: 17245108]
- Audeh MW, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. *Lancet*. 2010; 376:245–251. [PubMed: 20609468]
- Barker CA, Powell SN. Enhancing radiotherapy through a greater understanding of homologous recombination. *Seminars in radiation oncology*. 2010; 20:267–273 e3. [PubMed: 20832019]
- Barnes DE, et al. Targeted disruption of the gene encoding DNA ligase IV leads to lethality in embryonic mice. *Current biology : CB*. 1998; 8:1395–1398. [PubMed: 9889105]
- Baselga J, et al. Phase II trial of pertuzumab and trastuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer that progressed during prior trastuzumab therapy. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010; 28:1138–1144. [PubMed: 20124182]
- Bassing CH, et al. Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:8173–8178. [PubMed: 12034884]
- Baumann P, West SC. DNA end-joining catalyzed by human cell-free extracts. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95:14066–14070. [PubMed: 9826654]
- Bertocci B, et al. Nonoverlapping functions of DNA polymerases mu, lambda, and terminal deoxynucleotidyltransferase during immunoglobulin V(D)J recombination in vivo. *Immunity*. 2006; 25:31–41. [PubMed: 16860755]
- Block WD, et al. Autophosphorylation-dependent remodeling of the DNA-dependent protein kinase catalytic subunit regulates ligation of DNA ends. *Nucleic acids research*. 2004; 32:4351–4357. [PubMed: 15314205]
- Blumenschein GR Jr, et al. Phase II study of cetuximab in combination with chemoradiation in patients with stage IIIA/B non-small- cell lung cancer: RTOG 0324. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011; 29:2312–2318. [PubMed: 21555682]
- Blunt T, et al. Identification of a nonsense mutation in the carboxyl-terminal region of DNA-dependent protein kinase catalytic subunit in the scid mouse. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93:10285–10290. [PubMed: 8816792]
- Bonner JA, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *The New England journal of medicine*. 2006; 354:567–578. [PubMed: 16467544]
- Bonner JA, et al. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. *The lancet oncology*. 2010; 11:21–28. [PubMed: 19897418]
- Boone JJ, et al. Involvement of the HER2 pathway in repair of DNA damage produced by chemotherapeutic agents. *Molecular cancer therapeutics*. 2009; 8:3015–3023. [PubMed: 19887555]
- Bozulic L, et al. PKBalpha/Akt1 acts downstream of DNA-PK in the DNA double-strand break response and promotes survival. *Molecular cell*. 2008; 30:203–213. [PubMed: 18439899]
- Bupp JM, et al. Telomere anchoring at the nuclear periphery requires the budding yeast Sad1-UNC-84 domain protein Mps3. *The Journal of cell biology*. 2007; 179:845–854. [PubMed: 18039933]

- Burma S, et al. Role of non-homologous end joining (NHEJ) in maintaining genomic integrity. DNA repair. 2006; 5:1042–1048. [PubMed: 16822724]
- Burma S, et al. ATM phosphorylates histone H2AX in response to DNA double-strand breaks. The Journal of biological chemistry. 2001; 276:42462–42467. [PubMed: 11571274]
- Calsou P, et al. The DNA-dependent protein kinase catalytic activity regulates DNA end processing by means of Ku entry into DNA. The Journal of biological chemistry. 1999; 274:7848–7856. [PubMed: 10075677]
- Caponigro F, et al. A phase I/II trial of gefitinib and radiotherapy in patients with locally advanced inoperable squamous cell carcinoma of the head and neck. Anti-cancer drugs. 2008; 19:739–744. [PubMed: 18594217]
- Celeste A, et al. Genomic instability in mice lacking histone H2AX. Science. 2002; 296:922–927. [PubMed: 11934988]
- Chan DW, et al. Autophosphorylation of the DNA-dependent protein kinase catalytic subunit is required for rejoining of DNA double-strand breaks. Genes & development. 2002; 16:2333–2338. [PubMed: 12231622]
- Chan DW, Lees-Miller SP. The DNA-dependent protein kinase is inactivated by autophosphorylation of the catalytic subunit. The Journal of biological chemistry. 1996; 271:8936–8941. [PubMed: 8621537]
- Chen L, et al. Ataxia-telangiectasia-mutated dependent phosphorylation of Artemis in response to DNA damage. Cancer science. 2005; 96:134–141. [PubMed: 15723659]
- Chinnaiyan P, et al. Mechanisms of enhanced radiation response following epidermal growth factor receptor signaling inhibition by erlotinib (Tarceva). Cancer research. 2005; 65:3328–3335. [PubMed: 15833866]
- Contessa JN, et al. The inducible expression of dominant-negative epidermal growth factor receptor-CD533 results in radiosensitization of human mammary carcinoma cells. Clinical cancer research : an official journal of the American Association for Cancer Research. 1999; 5:405–411. [PubMed: 10037190]
- Cook PJ, et al. Tyrosine dephosphorylation of H2AX modulates apoptosis and survival decisions. Nature. 2009; 458:591–596. [PubMed: 19234442]
- Cortes J, et al. Pertuzumab monotherapy after trastuzumab-based treatment and subsequent reintroduction of trastuzumab: activity and tolerability in patients with advanced human epidermal growth factor receptor 2-positive breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012; 30:1594–1600. [PubMed: 22393084]
- Costantini S, et al. Interaction of the Ku heterodimer with the DNA ligase IV/Xrcc4 complex and its regulation by DNA-PK. DNA repair. 2007; 6:712–722. [PubMed: 17241822]
- Critchlow SE, et al. Mammalian DNA double-strand break repair protein XRCC4 interacts with DNA ligase IV. Current biology : CB. 1997; 7:588–598. [PubMed: 9259561]
- Cui X, et al. Autophosphorylation of DNA-dependent protein kinase regulates DNA end processing and may also alter double-strand break repair pathway choice. Molecular and cellular biology. 2005; 25:10842–10852. [PubMed: 16314509]
- Curran D, et al. Quality of life in head and neck cancer patients after treatment with high-dose radiotherapy alone or in combination with cetuximab. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007; 25:2191–2197. [PubMed: 17538164]
- Ding Q, et al. DNA-PKcs mutations in dogs and horses: allele frequency and association with neoplasia. Gene. 2002; 283:263–269. [PubMed: 11867233]
- Ding Q, et al. Autophosphorylation of the catalytic subunit of the DNA-dependent protein kinase is required for efficient end processing during DNA double-strand break repair. Molecular and cellular biology. 2003; 23:5836–5848. [PubMed: 12897153]
- Dittmann K, et al. Nuclear EGFR as novel therapeutic target: insights into nuclear translocation and function. Strahlentherapie und Onkologie : Organ der Deutschen Röntgengesellschaft ...[et al]. 2010; 186:1–6.
- Dominguez O, et al. DNA polymerase mu (Pol mu), homologous to TdT, could act as a DNA mutator in eukaryotic cells. The EMBO journal. 2000; 19:1731–1742. [PubMed: 10747040]

- Douglas P, et al. Identification of in vitro and in vivo phosphorylation sites in the catalytic subunit of the DNA-dependent protein kinase. *The Biochemical journal*. 2002; 368:243–251. [PubMed: 12186630]
- Drebin JA, et al. Monoclonal antibodies reactive with distinct domains of the neu oncogene-encoded p185 molecule exert synergistic anti-tumor effects in vivo. *Oncogene*. 1988; 2:273–277. [PubMed: 2451200]
- Drebin JA, et al. Down-modulation of an oncogene protein product and reversion of the transformed phenotype by monoclonal antibodies. *Cell*. 1985; 41:697–706. [PubMed: 2860972]
- Drebin JA, et al. Inhibition of tumor growth by a monoclonal antibody reactive with an oncogene-encoded tumor antigen. *Proceedings of the National Academy of Sciences of the United States of America*. 1986; 83:9129–9133. [PubMed: 3466178]
- Drouet J, et al. Interplay between Ku, Artemis, and the DNA-dependent protein kinase catalytic subunit at DNA ends. *The Journal of biological chemistry*. 2006; 281:27784–27793. [PubMed: 16857680]
- Eccles LJ, et al. Delayed repair of radiation induced clustered DNA damage: friend or foe? *Mutation research*. 2011; 711:134–141. [PubMed: 21130102]
- Falck J, et al. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature*. 2005; 434:605–611. [PubMed: 15758953]
- Ferguson KM, et al. EGF activates its receptor by removing interactions that autoinhibit ectodomain dimerization. *Molecular cell*. 2003; 11:507–517. [PubMed: 12620237]
- Foley, J., et al. At the crossroads: EGFR and PTHrP signaling in cancer-mediated diseases of bone. *Odontology / the Society of the Nippon Dental University*; 2012.
- Foray N, et al. Constitutive association of BRCA1 and c-Abl and its ATM-dependent disruption after irradiation. *Molecular and cellular biology*. 2002; 22:4020–4032. [PubMed: 12024016]
- Franchitto A, et al. The mammalian mismatch repair protein MSH2 is required for correct MRE11 and RAD51 relocalization and for efficient cell cycle arrest induced by ionizing radiation in G2 phase. *Oncogene*. 2003; 22:2110–2120. [PubMed: 12687013]
- Frank KM, et al. Late embryonic lethality and impaired V(D)J recombination in mice lacking DNA ligase IV. *Nature*. 1998; 396:173–177. [PubMed: 9823897]
- Fuller SJ, et al. ErbB receptors, their ligands, and the consequences of their activation and inhibition in the myocardium. *Journal of molecular and cellular cardiology*. 2008; 44:831–854. [PubMed: 18430438]
- Garcia-Diaz M, et al. DNA polymerase lambda, a novel DNA repair enzyme in human cells. *The Journal of biological chemistry*. 2002; 277:13184–13191. [PubMed: 11821417]
- Garrett TP, et al. Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha. *Cell*. 2002; 110:763–773. [PubMed: 12297049]
- Gell D, Jackson SP. Mapping of protein-protein interactions within the DNA-dependent protein kinase complex. *Nucleic acids research*. 1999; 27:3494–3502. [PubMed: 10446239]
- Golding SE, et al. Pro-survival AKT and ERK signaling from EGFR and mutant EGFRvIII enhances DNA double-strand break repair in human glioma cells. *Cancer biology & therapy*. 2009; 8:730–738. [PubMed: 19252415]
- Goodarzi AA, et al. DNA-PK autophosphorylation facilitates Artemis endonuclease activity. *The EMBO journal*. 2006; 25:3880–3889. [PubMed: 16874298]
- Gottlieb TM, Jackson SP. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. *Cell*. 1993; 72:131–142. [PubMed: 8422676]
- Graus-Porta D, et al. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *The EMBO journal*. 1997; 16:1647–1655. [PubMed: 9130710]
- Graus-Porta D, et al. Single-chain antibody-mediated intracellular retention of ErbB-2 impairs Neu differentiation factor and epidermal growth factor signaling. *Molecular and cellular biology*. 1995; 15:1182–1191. [PubMed: 7532277]
- Grawunder U, et al. Activity of DNA ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells. *Nature*. 1997; 388:492–495. [PubMed: 9242410]

- Grawunder U, et al. DNA ligase IV binds to XRCC4 via a motif located between rather than within its BRCT domains. *Current biology : CB*. 1998; 8:873–876. [PubMed: 9705934]
- Gregoire V, et al. Gefitinib plus cisplatin and radiotherapy in previously untreated head and neck squamous cell carcinoma: a phase II, randomized, double-blind, placebo-controlled study. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology*. 2011; 100:62–69. [PubMed: 21821303]
- Griffero F, et al. Different response of human glioma tumor-initiating cells to epidermal growth factor receptor kinase inhibitors. *The Journal of biological chemistry*. 2009; 284:7138–7148. [PubMed: 19147502]
- Gu J, et al. XRCC4:DNA ligase IV can ligate incompatible DNA ends and can ligate across gaps. *The EMBO journal*. 2007a; 26:1010–1023. [PubMed: 17290226]
- Gu J, et al. Single-stranded DNA ligation and XLF-stimulated incompatible DNA end ligation by the XRCC4-DNA ligase IV complex: influence of terminal DNA sequence. *Nucleic acids research*. 2007b; 35:5755–5762. [PubMed: 17717001]
- Gupta S, Meek K. The leucine rich region of DNA-PKcs contributes to its innate DNA affinity. *Nucleic acids research*. 2005; 33:6972–6981. [PubMed: 16340007]
- Hagan M, et al. Ionizing radiation causes a dose-dependent release of transforming growth factor alpha in vitro from irradiated xenografts and during palliative treatment of hormone-refractory prostate carcinoma. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2004; 10:5724–5731. [PubMed: 15355899]
- Hambardzumyan D, et al. PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma in vivo. *Genes & development*. 2008; 22:436–448. [PubMed: 18281460]
- Hentges P, et al. Evolutionary and functional conservation of the DNA non-homologous end-joining protein, XLF/Cernunnos. *The Journal of biological chemistry*. 2006; 281:37517–37526. [PubMed: 17038309]
- Heron DE, et al. Concurrent cetuximab with stereotactic body radiotherapy for recurrent squamous cell carcinoma of the head and neck: a single institution matched case-control study. *American journal of clinical oncology*. 2011; 34:165–172. [PubMed: 20686406]
- Holz C, et al. Epithelial-mesenchymal-transition induced by EGFR activation interferes with cell migration and response to irradiation and cetuximab in head and neck cancer cells. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology*. 2011; 101:158–164. [PubMed: 21665310]
- Horton JK, et al. Radiosensitization of chemotherapy-refractory, locally advanced or locally recurrent breast cancer with trastuzumab: a phase II trial. *International journal of radiation oncology, biology, physics*. 2010; 76:998–1004.
- Huang LC, et al. Sensitivity and selectivity of the DNA damage sensor responsible for activating p53-dependent G1 arrest. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93:4827–4832. [PubMed: 8643488]
- Huang SM, et al. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. *Cancer research*. 1999; 59:1935–1940. [PubMed: 10213503]
- Huang SM, et al. Modulation of radiation response and tumor-induced angiogenesis after epidermal growth factor receptor inhibition by ZD1839 (Iressa). *Cancer research*. 2002; 62:4300–4306. [PubMed: 12154033]
- Hurt CN, et al. SCOPE1: a randomised phase II/III multicentre clinical trial of definitive chemoradiation, with or without cetuximab, in carcinoma of the oesophagus. *BMC cancer*. 2011; 11:466. [PubMed: 22035459]
- Jaspersen SL, et al. Mps3p is a novel component of the yeast spindle pole body that interacts with the yeast centrin homologue Cdc31p. *The Journal of cell biology*. 2002; 159:945–956. [PubMed: 12486115]
- Jaspersen SL, et al. The Sad1-UNC-84 homology domain in Mps3 interacts with Mps2 to connect the spindle pole body with the nuclear envelope. *The Journal of cell biology*. 2006; 174:665–675. [PubMed: 16923827]

- Jatoi A, et al. A phase II study of cetuximab and radiation in elderly and/or poor performance status patients with locally advanced non-small- cell lung cancer (N0422). *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. 2010; 21:2040–2044. [PubMed: 20570832]
- Jensen AD, et al. Combined treatment of nonsmall cell lung cancer NSCLC stage III with intensity-modulated RT radiotherapy and cetuximab: the NEAR trial. *Cancer*. 2011; 117:2986–2994. [PubMed: 21264838]
- Jin S, et al. Binding of Ku and c-Abl at the kinase homology region of DNA-dependent protein kinase catalytic subunit. *The Journal of biological chemistry*. 1997; 272:24763–24766. [PubMed: 9312071]
- Joensuu G, et al. A phase I/II trial of gefitinib given concurrently with radiotherapy in patients with nonmetastatic prostate cancer. *International journal of radiation oncology, biology, physics*. 2010; 78:42–49.
- Junop MS, et al. Crystal structure of the Xrcc4 DNA repair protein and implications for end joining. *The EMBO journal*. 2000; 19:5962–5970. [PubMed: 11080143]
- Kang KB, et al. Gefitinib radiosensitizes stem-like glioma cells: inhibition of epidermal growth factor receptor-Akt-DNA-PK signaling, accompanied by inhibition of DNA double-strand break repair. *International journal of radiation oncology, biology, physics*. 2012; 83:e43–e52.
- Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. *Nature genetics*. 2001; 27:247–254. [PubMed: 11242102]
- Kim JS, et al. Independent and sequential recruitment of NHEJ and HR factors to DNA damage sites in mammalian cells. *The Journal of cell biology*. 2005; 170:341–347. [PubMed: 16061690]
- Kohda D, et al. A 40-kDa epidermal growth factor/transforming growth factor alpha-binding domain produced by limited proteolysis of the extracellular domain of the epidermal growth factor receptor. *The Journal of biological chemistry*. 1993; 268:1976–1981. [PubMed: 8420971]
- Kokai Y, et al. Synergistic interaction of p185c-neu and the EGF receptor leads to transformation of rodent fibroblasts. *Cell*. 1989; 58:287–292. [PubMed: 2568888]
- Kokunai T, et al. Overcoming of radioresistance in human gliomas by p21WAF1/CIP1 antisense oligonucleotide. *Journal of neuro-oncology*. 2001; 51:111–119. [PubMed: 11386407]
- Kriegs M, et al. The epidermal growth factor receptor modulates DNA double-strand break repair by regulating non-homologous end-joining. *DNA repair*. 2010; 9:889–897. [PubMed: 20615764]
- Kurimasa A, et al. Requirement for the kinase activity of human DNA-dependent protein kinase catalytic subunit in DNA strand break rejoining. *Molecular and cellular biology*. 1999; 19:3877–3884. [PubMed: 10207111]
- Lammering G, et al. Epidermal growth factor receptor as a genetic therapy target for carcinoma cell radiosensitization. *Journal of the National Cancer Institute*. 2001; 93:921–929. [PubMed: 11416113]
- Lax I, et al. Localization of a major receptor-binding domain for epidermal growth factor by affinity labeling. *Molecular and cellular biology*. 1988; 8:1831–1834. [PubMed: 3260004]
- Lee KK, et al. Lamin-dependent localization of UNC-84, a protein required for nuclear migration in *Caenorhabditis elegans*. *Molecular biology of the cell*. 2002; 13:892–901. [PubMed: 11907270]
- Lees-Miller SP, et al. Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line. *Science*. 1995; 267:1183–1185. [PubMed: 7855602]
- Lehman JA, et al. DNA-dependent conformational changes in the Ku heterodimer. *Biochemistry*. 2008; 47:4359–4368. [PubMed: 18355052]
- Lei K, et al. Inner Nuclear Envelope Proteins SUN1 and SUN2 Play a Prominent Role in the DNA Damage Response. *Current biology : CB*. 2012
- Li Y, et al. Crystal structure of human XLF/Cernunnos reveals unexpected differences from XRCC4 with implications for NHEJ. *The EMBO journal*. 2008; 27:290–300. [PubMed: 18046455]
- Li Z, et al. The XRCC4 gene encodes a novel protein involved in DNA double-strand break repair and V(D)J recombination. *Cell*. 1995; 83:1079–1089. [PubMed: 8548796]
- Liang K, et al. Sensitization of breast cancer cells to radiation by trastuzumab. *Molecular cancer therapeutics*. 2003; 2:1113–1120. [PubMed: 14617784]

- Lu H, et al. Length-dependent binding of human XLF to DNA and stimulation of XRCC4.DNA ligase IV activity. *The Journal of biological chemistry*. 2007; 282:11155–11162. [PubMed: 17317666]
- Ma Y, et al. The DNA-dependent protein kinase catalytic subunit phosphorylation sites in human Artemis. *The Journal of biological chemistry*. 2005a; 280:33839–33846. [PubMed: 16093244]
- Ma Y, et al. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell*. 2002; 108:781–794. [PubMed: 11955432]
- Ma Y, et al. The Artemis:DNA-PKcs endonuclease cleaves DNA loops, flaps, and gaps. *DNA repair*. 2005b; 4:845–851. [PubMed: 15936993]
- Magnander K, Elmroth K. Biological consequences of formation and repair of complex DNA damage. *Cancer letters*. 2012
- Mahajan KN, et al. Association of DNA polymerase mu (pol mu) with Ku and ligase IV: role for pol mu in end-joining double-strand break repair. *Molecular and cellular biology*. 2002; 22:5194–5202. [PubMed: 12077346]
- Mahaney BL, et al. Repair of ionizing radiation-induced DNA doublestrand breaks by non-homologous end-joining. *The Biochemical journal*. 2009; 417:639–650. [PubMed: 19133841]
- Mari PO, et al. Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:18597–18602. [PubMed: 17124166]
- Martin SA, et al. Therapeutic targeting of the DNA mismatch repair pathway. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2010; 16:5107–5113. [PubMed: 20823149]
- Masuda Y, Kamiya K. Molecular nature of radiation injury and DNA repair disorders associated with radiosensitivity. *International journal of hematology*. 2012; 95:239–245. [PubMed: 22351161]
- Meek K, et al. trans Autophosphorylation at DNA-dependent protein kinase's two major autophosphorylation site clusters facilitates end processing but not end joining. *Molecular and cellular biology*. 2007; 27:3881–3890. [PubMed: 17353268]
- Merkle D, et al. The DNA-dependent protein kinase interacts with DNA to form a protein-DNA complex that is disrupted by phosphorylation. *Biochemistry*. 2002; 41:12706–12714. [PubMed: 12379113]
- Minjgee M, et al. K-RAS(V12) induces autocrine production of EGFR ligands and mediates radioresistance through EGFR-dependent Akt signaling and activation of DNA-PKcs. *International journal of radiation oncology, biology, physics*. 2011; 81:1506–1514.
- Modesti M, et al. DNA binding of Xrcc4 protein is associated with V(D)J recombination but not with stimulation of DNA ligase IV activity. *The EMBO journal*. 1999; 18:2008–2018. [PubMed: 10202163]
- Moon AF, et al. The X family portrait: structural insights into biological functions of X family polymerases. *DNA repair*. 2007; 6:1709–1725. [PubMed: 17631059]
- Moshous D, et al. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell*. 2001; 105:177–186. [PubMed: 11336668]
- Mukherjee B, et al. EGFRvIII and DNA double-strand break repair: a molecular mechanism for radioresistance in glioblastoma. *Cancer research*. 2009; 69:4252–4259. [PubMed: 19435898]
- Murray MM, et al. Role played by BRCA1 in transcriptional regulation in response to therapy. *Biochemical Society transactions*. 2007; 35:1342–1346. [PubMed: 17956347]
- Nagai S, et al. Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science*. 2008; 322:597–602. [PubMed: 18948542]
- Nick McElhinny SA, et al. Ku recruits the XRCC4-ligase IV complex to DNA ends. *Molecular and cellular biology*. 2000; 20:2996–3003. [PubMed: 10757784]
- Nishikawa S, et al. Nep98p is a component of the yeast spindle pole body and essential for nuclear division and fusion. *The Journal of biological chemistry*. 2003; 278:9938–9943. [PubMed: 12493774]
- No M, et al. Targeting HER2 signaling pathway for radiosensitization: alternative strategy for therapeutic resistance. *Cancer biology & therapy*. 2009; 8:2351–2361. [PubMed: 19923913]

- O'Driscoll M, et al. DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. *Molecular cell*. 2001; 8:1175–1185. [PubMed: 11779494]
- O'Rourke DM, et al. Conversion of a radioresistant phenotype to a more sensitive one by disabling erbB receptor signaling in human cancer cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1998a; 95:10842–10847. [PubMed: 9724792]
- O'Rourke DM, et al. Inhibition of a naturally occurring EGFR oncoprotein by the p185neu ectodomain: implications for subdomain contributions to receptor assembly. *Oncogene*. 1998b; 16:1197–1207. [PubMed: 9528862]
- O'Rourke DM, et al. Trans receptor inhibition of human glioblastoma cells by erbB family ectodomains. *Proceedings of the National Academy of Sciences of the United States of America*. 1997; 94:3250–3255. [PubMed: 9096379]
- Ogiso H, et al. Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains. *Cell*. 2002; 110:775–787. [PubMed: 12297050]
- Oza P, et al. Mechanisms that regulate localization of a DNA doublestrand break to the nuclear periphery. *Genes & development*. 2009; 23:912–927. [PubMed: 19390086]
- Park BW, et al. Rationally designed anti-HER2/neu peptide mimetic disables P185HER2/neu tyrosine kinases in vitro and in vivo. *Nature biotechnology*. 2000; 18:194–198.
- Park J, et al. DNA-dependent protein kinase-mediated phosphorylation of protein kinase B requires a specific recognition sequence in the Cterminal hydrophobic motif. *The Journal of biological chemistry*. 2009; 284:6169–6174. [PubMed: 19144640]
- Pickhard AC, et al. Inhibition of radiation induced migration of human head and neck squamous cell carcinoma cells by blocking of EGF receptor pathways. *BMC cancer*. 2011; 11:388. [PubMed: 21896192]
- Pietras RJ, et al. Monoclonal antibody to HER-2/neureceptor modulates repair of radiation-induced DNA damage and enhances radiosensitivity of human breast cancer cells overexpressing this oncogene. *Cancer research*. 1999; 59:1347–1355. [PubMed: 10096569]
- Poinsignon C, et al. The metallo-beta-lactamase/beta-CASP domain of Artemis constitutes the catalytic core for V(D)J recombination. *The Journal of experimental medicine*. 2004; 199:315–321. [PubMed: 14744996]
- Portera CC, et al. Cardiac toxicity and efficacy of trastuzumab combined with pertuzumab in patients with [corrected] human epidermal growth factor receptor 2-positive metastatic breast cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2008; 14:2710–2716. [PubMed: 18451236]
- Povirk LF, et al. Processing of 3'-phosphoglycolate-terminated DNA double strand breaks by Artemis nuclease. *The Journal of biological chemistry*. 2007; 282:3547–3558. [PubMed: 17121861]
- Qian X, et al. Heterodimerization of epidermal growth factor receptor and wild-type or kinase-deficient Neu: a mechanism of interreceptor kinase activation and transphosphorylation. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91:1500–1504. [PubMed: 7509075]
- Reddy YV, et al. Non-homologous end joining requires that the DNA-PK complex undergo an autophosphorylation-dependent rearrangement at DNA ends. *The Journal of biological chemistry*. 2004; 279:39408–39413. [PubMed: 15258142]
- Riballo E, et al. Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Current biology : CB*. 1999; 9:699–702. [PubMed: 10395545]
- Riballo E, et al. A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci. *Molecular cell*. 2004; 16:715–724. [PubMed: 15574327]
- Riemsma R, et al. Systematic review of lapatinib in combination with letrozole compared with other first line treatments for hormone receptor positive (HR+) and HER2+ advanced or metastatic breast cancer (MBC). *Current medical research and opinion*. 2012
- Rodriguez-Rocha H, et al. DNA damage and autophagy. *Mutation research*. 2011; 711:158–166. [PubMed: 21419786]
- Roepstorff K, et al. Endocytic downregulation of ErbB receptors: mechanisms and relevance in cancer. *Histochemistry and cell biology*. 2008; 129:563–578. [PubMed: 18288481]

- Rogakou EP, et al. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *The Journal of biological chemistry*. 1998; 273:5858–5868. [PubMed: 9488723]
- Rooney S, et al. Leaky Scid phenotype associated with defective V(D)J coding end processing in Artemis-deficient mice. *Molecular cell*. 2002; 10:1379–1390. [PubMed: 12504013]
- Schober H, et al. Yeast telomerase and the SUN domain protein Mps3 anchor telomeres and repress subtelomeric recombination. *Genes & development*. 2009; 23:928–938. [PubMed: 19390087]
- Shin EK, et al. A kinase-negative mutation of DNA-PK(CS) in equine SCID results in defective coding and signal joint formation. *Journal of immunology*. 1997; 158:3565–3569.
- Shintani S, et al. Enhancement of tumor radioresponse by combined treatment with gefitinib (Iressa, ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor, is accompanied by inhibition of DNA damage repair and cell growth in oral cancer. *International journal of cancer. Journal international du cancer*. 2003; 107:1030–1037. [PubMed: 14601066]
- Sibanda BL, et al. Crystal structure of DNA-PKcs reveals a large open-ring cradle comprised of HEAT repeats. *Nature*. 2010; 463:118–121. [PubMed: 20023628]
- Singh N, et al. Dual recognition of phosphoserine and phosphotyrosine in histone variant H2A.X by DNA damage response protein MCPH1. *Proceedings of the National Academy of Sciences of the United States of America*. 2012
- Singleton BK, et al. The C terminus of Ku80 activates the DNA-dependent protein kinase catalytic subunit. *Molecular and cellular biology*. 1999; 19:3267–3277. [PubMed: 10207052]
- Skvortsova I, et al. Epithelial-to-mesenchymal transition and c-myc expression are the determinants of cetuximab-induced enhancement of squamous cell carcinoma radioresponse. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology*. 2010; 96:108–115. [PubMed: 20451273]
- Smyth EC, Cunningham D. Targeted therapy for gastric cancer. *Current treatment options in oncology*. 2012; 13:377–389. [PubMed: 22552927]
- Soeda A, et al. Epidermal growth factor plays a crucial role in mitogenic regulation of human brain tumor stem cells. *The Journal of biological chemistry*. 2008; 283:10958–10966. [PubMed: 18292095]
- Solomon B, et al. EGFR blockade with ZD1839 ("Iressa") potentiates the antitumor effects of single and multiple fractions of ionizing radiation in human A431 squamous cell carcinoma. Epidermal growth factor receptor. *International journal of radiation oncology, biology, physics*. 2003; 55:713–723.
- Sosa BA, et al. LINC Complexes Form by Binding of Three KASH Peptides to Domain Interfaces of Trimeric SUN Proteins. *Cell*. 2012; 149:1035–1047. [PubMed: 22632968]
- Soubeyrand S, et al. Artemis phosphorylated by DNA-dependent protein kinase associates preferentially with discrete regions of chromatin. *Journal of molecular biology*. 2006; 358:1200–1211. [PubMed: 16600297]
- Soubeyrand S, et al. Threonines 2638/2647 in DNA-PK are essential for cellular resistance to ionizing radiation. *Cancer research*. 2003; 63:1198–1201. [PubMed: 12649176]
- Sterr DA, Han M. Role of ANC-1 in tethering nuclei to the actin cytoskeleton. *Science*. 2002; 298:406–409. [PubMed: 12169658]
- Stea B, et al. Time and dose-dependent radiosensitization of the glioblastoma multiforme U251 cells by the EGF receptor tyrosine kinase inhibitor ZD1839 ('Iressa'). *Cancer letters*. 2003; 202:43–51. [PubMed: 14643025]
- Stucki M, et al. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. *Cell*. 2005; 123:1213–1226. [PubMed: 16377563]
- Suwa A, et al. DNA-dependent protein kinase (Ku protein-p350 complex) assembles on double-stranded DNA. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91:6904–6908. [PubMed: 8041718]
- Tanaka T, et al. Gefitinib radiosensitizes non-small cell lung cancer cells by suppressing cellular DNA repair capacity. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2008; 14:1266–1273. [PubMed: 18281562]
- Taylor TE, et al. Targeting EGFR for treatment of glioblastoma: molecular basis to overcome resistance. *Current cancer drug targets*. 2012; 12:197–209. [PubMed: 22268382]

- Toulany M, et al. Blockage of epidermal growth factor receptor-phosphatidylinositol 3-kinase-AKT signaling increases radiosensitivity of K-RAS mutated human tumor cells in vitro by affecting DNA repair. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2006; 12:4119–4126. [PubMed: 16818713]
- Toulany M, et al. Targeting of AKT1 enhances radiation toxicity of human tumor cells by inhibiting DNA-PKcs-dependent DNA double-strand break repair. *Molecular cancer therapeutics*. 2008; 7:1772–1781. [PubMed: 18644989]
- Toulany M, et al. Akt Promotes Post-Irradiation Survival of Human Tumor Cells through Initiation, Progression, and Termination of DNA-PKcs- Dependent DNA Double-Strand Break Repair. *Molecular cancer research : MCR*. 2012
- Toulany M, et al. ErbB2 expression through heterodimerization with erbB1 is necessary for ionizing radiation- but not EGF-induced activation of Akt survival pathway. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology*. 2010; 97:338–345. [PubMed: 20347166]
- Tsai CJ, et al. Cernunnos/XLF promotes the ligation of mismatched and noncohesive DNA ends. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:7851–7856. [PubMed: 17470781]
- Tutt A, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. *Lancet*. 2010; 376:235–244. [PubMed: 20609467]
- Walker JR, et al. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature*. 2001; 412:607–614. [PubMed: 11493912]
- Wallasch C, et al. Heregulin-dependent regulation of HER2/neu oncogenic signaling by heterodimerization with HER3. *The EMBO journal*. 1995; 14:4267–4275. [PubMed: 7556068]
- Wang M, et al. EGF receptor inhibition radiosensitizes NSCLC cells by inducing senescence in cells sustaining DNA double-strand breaks. *Cancer research*. 2011; 71:6261–6269. [PubMed: 21852385]
- Wang Q, et al. Adenosine nucleotide modulates the physical interaction between hMSH2 and BRCA1. *Oncogene*. 2001; 20:4640–4649. [PubMed: 11498787]
- Wang Q, et al. BRCA1 binds c-Myc and inhibits its transcriptional and transforming activity in cells. *Oncogene*. 1998; 17:1939–1948. [PubMed: 9788437]
- Wang W, et al. Structural insights into SUN-KASH complexes across the nuclear envelope. *Cell research*. 2012
- Wang Y, et al. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes & development*. 2000; 14:927–939. [PubMed: 10783165]
- Ward IM, Chen J. Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress. *The Journal of biological chemistry*. 2001; 276:47759–47762. [PubMed: 11673449]
- Weidhaas JB, et al. A conserved RAS/mitogen-activated protein kinase pathway regulates DNA damage-induced cell death postirradiation in *Drosophila*. *Cancer research*. 2006; 66:10434–10438. [PubMed: 17079464]
- Weterings E, et al. The role of DNA dependent protein kinase in synopsis of DNA ends. *Nucleic acids research*. 2003; 31:7238–7246. [PubMed: 14654699]
- Weterings E, et al. The Ku80 carboxy terminus stimulates joining and artemis-mediated processing of DNA ends. *Molecular and cellular biology*. 2009; 29:1134–1142. [PubMed: 19103741]
- Yano K, Chen DJ. Live cell imaging of XLF and XRCC4 reveals a novel view of protein assembly in the non-homologous end-joining pathway. *Cell cycle*. 2008; 7:1321–1325. [PubMed: 18418068]
- Yoo S, Dynan WS. Geometry of a complex formed by double strand break repair proteins at a single DNA end: recruitment of DNA-PKcs induces inward translocation of Ku protein. *Nucleic acids research*. 1999; 27:4679–4686. [PubMed: 10572166]
- Zhang HT, et al. Relationship of p21^BBRCA1 to tyrosine kinase signaling pathways and the cell cycle in normal and transformed cells. *Oncogene*. 1997; 14:2863–2869. [PubMed: 9205094]

- Zhang X, et al. SUN1/2 and Syne/Nesprin-1/2 complexes connect centrosome to the nucleus during neurogenesis and neuronal migration in mice. *Neuron*. 2009; 64:173–187. [PubMed: 19874786]
- Zhang X, et al. Artemis is a phosphorylation target of ATM and ATR and is involved in the G2/M DNA damage checkpoint response. *Molecular and cellular biology*. 2004; 24:9207–9220. [PubMed: 15456891]
- Zhou H, et al. Effects of the EGFR/HER2 kinase inhibitor GW572016 on EGFR- and HER2-overexpressing breast cancer cell line proliferation, radiosensitization, and resistance. *International journal of radiation oncology, biology, physics*. 2004; 58:344–352.
- Zhou Z, et al. Structure of Sad1-UNC84 homology (SUN) domain defines features of molecular bridge in nuclear envelope. *The Journal of biological chemistry*. 2012; 287:5317–5326. [PubMed: 22170055]

Figure 1. Structure of the SUN Domain Monomer and the SUN-KASH Complex

(A) Views of SUN domain monomer from two different directions. The left panel depicts the SUN domain as a single protomer while the right panel depicts the SUN domain in the homotrimeric form. The center panel illustrates where the SUN2 trimer integrates into the inner nuclear membrane (INM). This figure was adapted from (Zhou et al., 2012). (B) Views of the SUN-KASH complex from two different directions. The left panel shows the interaction between one SUN protomer (yellow) and one KASH peptide (green) while the right panel demonstrates the trimeric SUN-KASH complex. In the right panel, the three protomers of the SUN homotrimer are colored yellow, blue, and orange and the three KASH peptides are colored magenta, green, and red. The center panel illustrates how the SUN-KASH complex interacts in the perinuclear space with the SUN and KASH domains integrated into the INM and outer nuclear membrane (ONM), respectively. This figure was adapted from (Wang et al., 2012).

Figure 2. Proposed Role of SUN Proteins in the Radiation Mediated DNA Damage Response
 Radiation (A) can cause DNA damage (B). SUN proteins form a network to accommodate the repair for damaged DNAs (C). Insert: A model of the SUN2 network based on the trimeric structure of SUN coiled-coil domain and inter-trimer interaction (viewed from the inside of the nucleus).