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Review

ERK kinases modulate the activation of PI3 kinase related kinases (PIKKs) in DNA damage response

Xiaozeng Lin[1,2,3], Judy Yan[1,2,3] and Damu Tang[1,2,3]

[1]Division of Nephrology, Department of Medicine, McMaster University, [2]Father Sean O'Sullivan Research Institute, [3]the Hamilton Center for Kidney Research, St. Joseph's Hospital, Hamilton, Ontario, Canada

Summary. DNA damage response (DDR) is the critical surveillance mechanism in maintaining genome integrity. The mechanism activates checkpoints to prevent cell cycle progression in the presence of DNA lesions, and mediates lesion repair. DDR is coordinated by three apical PI3 kinase related kinases (PIKKs), including ataxia-telangiectasia mutated (ATM), ATM- and Rad3-related (ATR), and DNA-PKcs (the catalytic subunit of the DNA dependent protein kinase). These kinases are activated in response to specific DNA damage or lesions, resulting in checkpoint activation and DNA lesion repair. While it is clear that the pathways of ATM, ATR, and DNA-PK are the core components of DDR, there is accumulating evidence revealing the involvement of other cellular pathways in regulating DDR; this is in line with the concept that in addition to being a nuclear event DDR is also a cellular process. One of these pathways is the extracellular signal-regulated kinase (ERK) MAPK (mitogen-activated protein kinase) pathway. ERK is a converging point of multiple signal transduction pathways involved in cell proliferation, differentiation, and apoptosis. Adding to this list of pathways is the recent development of ERK in DDR. The ERK kinases (ERK1 and ERK2) contribute to the proper execution of DDR in terms of checkpoint activation and the repair of DNA lesions. This review summarizes the contributions of ERK to DDR with emphasis on the relationship of ERK kinases with the activation of ATM, ATR, and DNA-PKcs.

Key words: ERK kinases, DNA damage response, ATM, ATR, DNA-PKcs

Offprint requests to: Damu Tang, T3310, St. Joseph's Hospital, 50 Charlton Ave East Hamilton, Ontario, Canada. e-mail: damut @mcmaster.ca

Introduction

Eukaryotic cells use a complex system, DDR, to maintain genome integrity and to ensure the accurate transmission of genetic information to the next generation of cells. This is largely achieved by the activation of ATM, ATR, and DNA-PK, and their pathways. Double stranded DNA breaks (DSBs) activate ATM and DNA-PKcs. ATM initiates checkpoint activation and coordinates DSB repair by homologous recombination (HR) (Zhou and Elledge, 2000; Smith et al., 2010). Additionally, activation of DNA-PKcs plays an essential role in the repair of DSBs by non-homologous end joining (NHEJ) (Ding et al., 2003; Mahaney et al., 2009). ATR is primarily activated by replication protein A (RPA)-coated single-stranded DNA (ssDNA) (Costanzo et al., 2003; Zou and Elledge, 2003), although other DNA lesions, including DSBs, base adducts and crosslinks, also activate ATR (Cimprich and Cortez, 2008). ATR plays essential roles in checkpoint activation, stabilization of replication forks, and promoting DNA damage repair (Lopez-Contreras and Fernandez-Capetillo, 2010; Flynn and Zou, 2011). Despite these apical PIKKs being critical to DDR, the mechanisms governing their activation remain incompletely understood. Consistent with ATM, ATR and DNA-PKcs being members of PIKKs, they share the typical structural features of PIKKs, including the FRAP-ATM-TRRAP (FAT), kinase, and C-terminal FAT (FATC) domains (Cimprich and Cortez, 2008; Lempiäinen and Halazonetis, 2009). These structural similarities are responsible for the common mechanisms used to activate ATM, ATR, and DNA-PKcs; these kinases are activated by interactions with specific proteins at the unique DNA lesions. ATM activation requires the association with the MRE11/RAD50/NBS1

(MRN) complex at the DSBs via direct binding to NBS1 (Difilippantonio et al., 2005; Falck et al., 2005). ATR activation takes place at RPA-coated ssDNA through the interaction of the ATR-ATRIP (ATR-interacting protein) complex with TOPBP1 (Cimprich and Cortez, 2008; Burrows and Elledge, 2008). Activation of DNA-PKcs is mediated via binding to the Ku70/80 heterodimer and DSBs (Gottlieb and Jackson, 1993; Suwa et al., 1994; Uematsu et al., 2007). In addition to the aforementioned DNA lesions and protein binding partners, the activation of ATM, ATR, and DNA-PKcs is also affected by other cellular pathways.

One of the pathways is the ERK MAP kinases. ERK consists of two highly related kinases, ERK1 and ERK2; their activation is mediated by two conserved MAPK kinases, MEK1 and MEK2 which in turn are activated by Raf (MAPK kinase kinase) (Kolch, 2000; Krepinsky et al., 2002). MEK-mediated ERK activation is facilitated by the scaffold protein KSP1 (the kinase suppressor of Ras1); the association with KSP1 enhances MEK-mediated phosphorylation of ERK at Thr183 and Tyr185, an event that is required for ERK activation (Kolch, 2000; Peyssonnaux and Eychène, 2001; Kolch, 2005). ERK activity plays a role in multiple cellular processes, including proliferation, differentiation, apoptosis, and DDR (Kolch, 2000; Cowley et al., 1994; Chen et al., 2005; Wei et al., 2011a). In this review, we will briefly discuss the evidence demonstrating the involvement of ERK kinases in DDR, and focus on the interplay between ERK kinases and the PIKKs (ATM, ATM, and DNA-PKcs). Finally, we will suggest some critical future research.

ERK kinases contribute to DDR

In the last decade, ERK activation was repeatedly demonstrated by numerous investigators in a variety of cell lines in response to multiple DNA lesions induced by different genotoxic reagents. Etoposide, adriamycin, UV, ionizing radiation (IR), hydroxyurea (HU), mitomycin C, and cisplatin have all been shown to induce ERK activation in mouse embryonic fibroblasts (MEFs), NIH3T3, IMR90, MCF7, U87, H9c2, HeLa, A2780, HEI-OCI, T98G, and U2OS cells (Lee et al., 2000; Wang et al., 2000; Persons et al., 2000; Tang et al., 2002; Wu et al., 2006; Golding et al., 2007; So et al., 2007; Yan et al., 2007; Liu et al., 2008; Saegusa et al., 2008; Razidlo et al., 2009; Hamdi et al., 2008; Tentner et al., 2012). ERK activity subsequently modulates DNA damage-induced cell cycle arrest and apoptosis. Inhibition of MEK-mediated ERK activation by using the well-established MEK inhibitors, PD98059 and U0126, prevented etoposide-induced G2/M arrest in NIH3T3 and IR-initiated G2/M arrest in MCF7 cells (Tang et al., 2002; Yan et al., 2007). Knockdown of both ERK1 and ERK2 reduced IR-induced G2/M arrest in MCF7 cells and knockdown of either ERK1 or ERK2 was sufficient to abolish G2/M arrest in MCF7 cells in response to etoposide treatment (Wei et al., 2010). In addition to G2/M arrest, DNA damage-induced S arrest was also facilitated by ERK activity. While the constitutively active MEK1Q56P sensitized HU-induced S phase arrest in MCF7 cells, inhibition of ERK activation by a dominant-negative MEK1K97M and MEK inhibitors (PD98059 and U0126) attenuated HU-initiated S phase arrest in MCF7 cells (Wu et al., 2006). Furthermore, knockdown of either ERK1 or ERK2 attenuated the accumulation of MCF7 cells in S phase in response to HU exposure (Wei at al., 2011b). ERK activity also promotes DNA damage-induced G1/S arrest (Tentner et al., 2012). Additionally, the MEK-ERK pathway plays a critical role in IR-induced cell cycle arrest in Drosophilia embryos (Moglia et al., 2006). Taken together, accumulating evidence reveals that DNA damage functionally activates the ERK kinases.

In supporting the above observations, ERK activity also sensitizes DNA damage-initiated apoptosis. While etoposide potently induced apoptosis in NIH3T3 cells, enforced ERK activation by using the constitutively active MEK1Q56P enhanced etoposide-induced apoptosis in NIH3T3 cells. Conversely, inhibition of ERK activation by PD98059 reduced cell death. PD98059 also enhanced the long-term survival of NIH3T3 cells when treated with either UV radiation or adriamycin (Tang et al., 2002). ERK activity promotes MMC-induced apoptosis in neurons (Lee and Kim, 2007). Cisplatin treatment induces ERK activation and apoptosis in a variety of cell lines; inhibition of ERK activation robustly prevents cisplatin-induced cell death in HepG2 and Huh7 hepatocarcinoma, A172 glioma, Saos-2 osteosarcoma, and HeLa cervical cancer cells (Wang et al., 2000; Woessmann et al., 2002; Alexia et al., 2004; Choi et al., 2004). Cisplatin and doxorubicin-induced apoptosis in HeLa and H9c2 cells was in part attributable to the upregulation of pro-apoptotic Bcl-2 members Noxa and Bax, respectively, a process that is mediated by ERK activity (Sheridan et al., 2010; Liu et al., 2008). Furthermore, in *C. elegans* IR induces apoptosis in germ cells via activation of MPK-1, the C. elegans' ERK orthologue, and MPK-1 regulates the C. elegans p53 family member CEP-1-mediated apoptosis (Rutkowski et al., 2011). Collectively, while ERK activity was reported to provide a survival advantage in genotoxic reagents-treated multiple myeloma and acute myelogenous leukemia cells (Dai et al., 2008; Nishioka et al., 2009), accumulating evidence demonstrates that ERK activity promotes apoptosis in a variety of DDR settings.

DNA damage activates ERK via complex mechanisms

While it is well documented that DNA damage induces ERK activation, the underlying mechanisms remain incompletely understood. In line with DDR being initiated from the nucleus and ATM being an apical kinase in DDR, adriamycin is unable to activate ERK in AT fibroblasts GM05823 (Tang et al., 2002) and DSB-induced ERK activation was inhibited by an ATM inhibitor KU-55933 (Khalil et al., 2011). Consistent with

these observations, caffeine, which can inhibit ATM, was able to reduce etoposide-induced ERK activation in human embryonic fibroblasts (Heo et al., 2012). Under overexpression conditions, wild type but not kinase-dead ATM formed complexes with active ERK and the formation of these complexes was significantly enhanced by etoposide treatment, suggesting that ATM kinase activity plays a direct role in ERK activation (Heo et al., 2012). It will be intriguing to see whether MEK exists in the ATM-ERK complex, which will shed light on how ATM activates ERK during DDR. Regardless of whether the ATM-ERK complex contains MEK, a large body of evidence demonstrated that DNA damage-induced ERK activation depends on MEK. In cultured cells, ERK activation in DDR is inhibited by MEK inhibitors PD98059 and U0126, and by dominant-negative MEK1K97M. In Drosophila embryos, the lack of MEK abolished DNA damage-mediated ERK activation (Moglia et al., 2006). On the other hand, DNA damage-induced ERK activation is sensitized by the constitutively active MEK1Q56P (Tang et al., 2002; Woessmann et al., 2002; Wu et al., 2006; Golding et al. 2007; Yan et al., 2007; Dai et al., 2008; Liu et al., 2008; Heo et al., 2012).

Although the canonical ERK activation is mediated by the MAPK kinase (MEK) and the MAPK kinase kinase (Raf) (Krepinsky et al., 2002), whether Raf is required for the activation of MEK-ERK remains unclear. It has been reported that ectopic expression of a dominant-negative Ras-N17 inhibited cisplatin-induced ERK activation in Saos-2 cells (Woessmann et al., 2002). As Ras signalling can activate the Raf-MEK-ERK pathway (Tarttaglia and Gelb, 2010), the observation suggested the involvement of Raf in ERK activation in DDR. In supporting this possibility, a dominant-negative Raf was found to inhibit etoposide, cisplatin, and camptothecin-induced ERK activation in human embryonic fibroblasts (Heo et al., 2012). However, in Drosophila embryos, DNA damage-induced activation of MEK-ERK is Raf-independent (Moglia et al., 2006), and inhibition of ATM activation reduced DNA damage-induced ERK but not Raf activation (Heo et al., 2012). Taken together, it is clear that MEK is essential in ERK activation during DDR, and what mediates MEK activation requires further investigation. It is possible that signals upstream of MEK are cell dependent. While DDR is primarily caused by DNA lesions inside the nucleus, DNA damage also induces extra-nuclear signaling events. In those cells where nuclear signals consist of the major DDR inputs, PIKKs may play a major role in MEK activation. In other cells where extra-nuclear signaling contributes to DDR, Raf may be activated by extra-nuclear events and thereby play a role in the activation of MEK-ERK.

ERK promotes checkpoint activation in DDR by facilitating ATM and ATR activation

Despite ATM and ATR being essential for checkpoint activation in DDR, mechanisms by which DNA damage induces ATM and ATR activation are not fully understood. This may be in part attributable to our limited knowledge in the involvement of other cellular systems in DNA damage-induced activation of ATM and ATR. In supporting this possibility, ERK activity was found to contribute to ATM and ATR activation.

It was reported that a MEK inhibitor PD98059 inhibited IR-induced ERK activation and reduced the formation of active ATM (S1981 phosphorylated ATM) nuclear foci (Golding et al., 2007). Consistent with ATM phosphorylating p53 at S15 (Zhou and Elledge, 2000), a variety of genotoxic reagents, including etoposide, cisplatin, camptothecin, and doxorubicin-induced p53 S15 phosphorylation was inhibited by PD98059 and U0126 (Persons et al., 2000; Heo et al., 2012; Liu et al., 2008). Furthermore, knockdown of either ERK1 or ERK2 reduced etoposide-induced phosphorylation of ATM at S1981 in MCF7 cells (Wei et al., 2010); S1981 phosphorylation induces the formation of active ATM monomers from inactive ATM dimers (Bakkenist and Kastan, 2003). In supporting that both ERK1 and ERK2 play a role in etoposide-induced ATM activation, knockdown of either ERK1 or ERK2 significantly reduced etoposide-induced nuclear foci of S1981-phosphorylated ATM, the phosphorylation of ATM targets (Chk2 T68 and p53 S15), and G2/M arrest in MCF7 cells (Wei et al., 2010). Additional evidence supporting the role of ERK in the activation of ATM-mediated G2/M checkpoints was a recent report demonstrating that Jaceosidin, a drug with anti-cancer activity, induces the ATM-mediated G2/M checkpoints via induction of ERK activation (Lee et al., 2013). While the underlying mechanisms responsible for ERK promoting ATM activation are not clear, evidence suggests that the complex of ATM/ERK contributes to ERK-facilitated ATM activation during DDR (Heo et al., 2012).

In addition to promoting ATM activation, ERK also facilitates ATR activation during DDR. IR induces ATR activation in MCF7 cells. The MEK inhibitor U0126 not only inhibited ERK activation but also reduced ATR activity, measured by an in vitro kinase assay, in IR-treated MCF7 cells (Yan et al., 2007). As U0126 at 50 μM, a dose commonly used in cells, has no effects on ATR kinase activity in an in vitro kinase assay using GST-p53 (residues 1-40) as the substrate (Wu et al., 2006), it is most likely that U0126 reduces IR-induced ATR activation by inhibiting ERK activation rather than directly inhibiting ATR kinase activity. In supporting this possibility, hydroxyurea (HU), a genotoxic reagent that primarily induces ATR activation (Segurado and Tercero, 2009), induces ATR nuclear foci, indicative of ATR activation, a process that is significantly reduced by U0126 (Wu et al., 2006). Phosphorylation of an ATR target p53 S15 was also reduced by U0126 in HU-treated MCF7 cells. Similarly, HU-induced ATR nuclear foci and p53 S15 phosphorylation are decreased by ectopic expression of a dominant-negative MEK1K97M.

Conversely, both events are enhanced in MCF7 cells expressing the constitutively active MEK1Q56P upon HU exposure (Wu et al., 2006). Furthermore, knockdown of either ERK1 or ERK2 significantly reduced HU-induced ATR nuclear foci, the phosphorylation of ATR targets (p53 S15 and Chk1 S345), and S phase arrest in MCF7 cells (Wei et al., 2011b). While knockdown of either ERK1 or ERK2 was effective in reducing HU-induced activation of the ATR pathway, knockdown of ERK2 seems more potent than knockdown of ERK1 (Wei et al., 2011b). Similar to the situation observed between ERK and ATM (Heo et al., 2012), ERK forms complexes with ATR and this interaction seems to be enhanced upon DNA damage (Yan et al., 2007). It is thus possible that the formation of this complex contributes to ERK-facilitated ATR activation.

Function of ERK in DSB repair and its impact on DNA-PKcs activation

While the contribution of ERK towards checkpoint activation in DDR has been extensively studied, its role in DSB repair remains less clear. By using an I-SceI-based HR assay in U87 cells, it was reported that both MEK inhibitors PD98059 and PD184252 reduced HR-mediated DSB repair (Golding et al., 2007), demonstrating that ERK kinases enhance HR. This is in line with reports showing that ERK facilitates DSB-induced ATM activation (Golding et al., 2007; Wei et al., 2010; Heo et al., 2012) and that ATM activity promotes HR (Smith et al., 2010; Wang et al., 2013). Collectively, evidence supports that ERK activity promotes HR-mediated DSB repair during DDR.

Under other settings, ERK also promotes NHEJ. By using I-SceI-based NHEJ reporter cell lines, EGFR signaling has been reported to enhance HR and NHEJ in U87 cells (Golding et al., 2009), and NHEJ in A549 cells (Kriegs et al., 2010). Inhibition of AKT and ERK activation using specific inhibitors reduced EGFR-stimulated NHEJ in U87 cells (Golding et al., 2009), while knockdown of ERK but not AKT decreased NHEJ in A549 cells (Kriegs et al., 2010). EGFR signaling-facilitated NHEJ might be cell dependent. In comparison to A549 cells, activation of EGFR only marginally enhanced NHEJ in H1299 cells (Kriegs et al., 2010).

In our recent investigation, we reported that ERK surprisingly reduced NHEJ in MCF7 cells (Wei et al., 2013). Etoposide at 10 μM induced a comparable amount of DSBs in MCF7 cells with and without ERK activation being inhibited, based on the levels of γH2AX, numbers of γH2AX foci, and DNA tail moment (comet assay) (Wei et al., 2013). However, the kinetics of γH2AX foci resolution and tail moment reduction, indicative of the dynamics of DSB repair, was significantly enhanced when ERK activation was inhibited by the MEK inhibitor U0126, the dominant-negative MEK1K97M, and knockdown of either ERK1 or ERK2 (Wei et al., 2013). Conversely, the repair of DSB (the removal of γH2AX foci and tail moment) was significantly delayed by the constitutively active MEK1Q56P (Wei et al., 2013). In line with NHEJ being preferentially used to repair DSBs in mammalian cells (Mladenow and Iliakis, 2011), by using a plasmid-based NHEJ assay both U0126 and knockdown of either ERK1 or ERK2 enhanced NHEJ (Wei et al., 2013). As activation of DNA-PKcs plays an essential role in NHEJ (Mahaney et al., 2009; Mladenow and Iliakis, 2011), we were able to show that inhibition of ERK activation by U0126, MEK1K97M, and knockdown of either ERK1 or ERK2 robustly elevated etoposide-induced phosphorylation of DNA-PKcs at S2056 (Wei et al., 2013). DNA-PKcs S2056 phosphorylation is widely used as a surrogate marker of DNA-PKcs activation (Douglas et al., 2007; Morris et al., 2011). On the other hand, ectopic expression of the constitutively active MEK1Q56P reduced etoposide-induced DNA-PKcs S2056 phosphorylation in MCF7 cells (Wei et al., 2013). Taken together, we provide compelling evidence that ERK activity inhibits DSB-induced DNA-PKcs activation, and thereby reduces NHEJ-mediated DSB repair.

In view of ERK-facilitating ATM and ATR activation in DDR, the observation is intriguing that ERK activity inhibits DSB-induced activation of DNA-PKcs (Wei et al., 2013). However, the observed inhibitory effects of ERK on DNA-PKcs are in line with our understanding of HR and NHEJ-mediated DSB repair. DSB is a highly toxic DNA lesion, and is mainly repaired by HR and NHEJ (Mahaney et al., 2009). In comparison to the error-free process of HR, NHEJ often results in mutations (Lieber, 2010). While regulation of DSB repair pathway choice remains unclear, recent developments show that HR and NHEJ are antagonistic to each other. The committing event is the resection of DNA ends for HR and the protection of DNA ends for NHEJ; the initiation of end resection prevents NHEJ and the end protection suppresses HR (Symington and Gautier, 2011). In fission yeast and vertebrates, a critical end resection protein CtIP (Ctp1 in yeast) is specifically expressed in S/G2 (Limbo et al., 2007; Buis et al., 2012); CDK2 via binding to MRE11 phosphorylates CtIP in S/G2 in S. cerevisiae and vertebrates, which promotes CtIP-mediated resection of DNA ends (Huertas et al., 2008; Buis et al., 2012). This mechanism contributes to the repression of HR in G1 and its activation in S/G2. Genetically, mouse embryonic stem cells deficient for *Ku70, XRCC4*, or *DNA-PKcs* (essential components of NHEJ), and *Ku70*$^{-/-}$ mouse embryonic fibroblasts display elevation in HR levels (Pierce et al., 2001; Bunting et al., 2012). BRCA1 and 53BP1 play critical roles in the commitment to HR and NHEJ, respectively, and one represses the other to ensure either HR or NHEJ process (Chapman et al., 2012). *BRCA1* deficiency results in defective HR in humans and mice, which leads to genome instability and tumorigenesis. Deletion of 53BP1 enhances resection of DSB ends, and increases HR in *BRCA1*$^{-/-}$ mice and thereby rescues *BRCA1*

deficiency-induced embryonic lethality, tumorigenesis, and chromosome abnormalities (Cao et al., 2009; Bouwman et al., 2010; Bunting et al., 2010). Collectively, genetic evidence clearly demonstrates the mutual repressive regulation between HR and NHEJ.

In view of this knowledge, it may require a revisit to whether ERK activity promotes both HR and NHEJ in the same cells under the same setting of DSB repair (Golding et al., 2009). On the other hand, the findings that ERK facilitates ATM activation, and suppresses DNA-PKcs activation in DDR are consistent with ERK's role in promoting HR and inhibiting NHEJ. This is further supported by the requirement of ATM kinase activity in recruiting CtIP to DSBs to initiate HR repair (You et al., 2009).

Future perspectives

While the contributions of ERK towards DDR have been repeatedly demonstrated over more than a decade, the significance of ERK in DDR regulation has not been fully recognized. This is largely attributable to the lack of physiological relevance of ERK to DDR, and to the limited knowledge on mechanisms responsible for ERK activation in DDR and for ERK-modulated activation of ATM, ATR, and DNA-PKcs.

Investigation of the physiological contributions of ERK to DDR has been a challenge due to the high level (84% identify) of homology between ERK1 and ERK2 as well as their seemingly redundant functions in vitro (Roskoski, 2012). However, in view of the recent development that knockdown of either ERK1 or ERK2 affected DNA damage-induced activation of ATM, ATR, and DNA-PKcs (Wei et al., 2010, 2011a,b, 2013), an effort should be made to determine the impact of ERK1 or ERK2 deficiency on DDR in mice. *ERK1*$^{-/-}$ mice are viable and fertile with defective thymocyte development (Pages et al., 1999), while *ERK2*$^{-/-}$ mice are embryonic lethal (Yao et al., 2003). By taking advantage of *ERK1*$^{-/-}$ mice, the impact of ERK1 deficiency on DDR induced by a variety of genotoxic reagents can be determined.

While it is clear that activation of ERK in DDR requires MEK activity, the upstream signals leading to MEK activation remain less clear. As DNA damage induces wild type but not kinase-dead ATM to form complexes with ERK (Heo et al., 2012), suggesting the requirement of ATM kinase activity in ERK activation, it will be critical to identify the components of the ATM-ERK complex. Will the complex contain MEK, the KSP1 adaptor protein that facilitates MEK-mediated ERK activation, or a MAPK kinase kinase? Which substrates does ATM phosphorylate to result in the activation of the MEK-ERK pathway?

On the other hand, will the ATM-ERK complex play a role in DSB-induced ATM activation? Activation of ATM is mediated by a direct binding to NBS1, leading to its association with the MRN complex and the subsequent recruitment of ATM to DSBs (Difilippantonio et al., 2005; Falck et al., 2005). As ERK activation contributes to the formation of S1918-phosphorylated ATM nuclear foci (Wei et al., 2010), it is a good possibility the ATM-ERK complex may also contain NBS1 or the MRN complex. Will ERK phosphorylate NBS1, the other components of the MRN complex, or ATM to facilitate ATM activation?

ATR activation requires independent recruitment of the ATR-ATRIP complex and TOPBP1 to RPA-coated ssDNA; TOPBP1 subsequently binds to the ATR-ATRIP complex, resulting in ATR activation; ERK forms complexes with ATR and this interaction seems to be enhanced upon DNA damage (Lee et al., 2007; Delacroix et al., 2007; Kumagai et al., 2006; Mordes et al., 2008). In response to DNA damage, ATR forms a complex with ERK (Yan et al., 2007); recruitment of ATR to DNA lesions is facilitated by ERK activity (Wei et al., 2011a,b). It is thus conceivable that ERK activity may promote the association of the ATR-ATRIP complex with RPA-coated ssDNA. ERK may also facilitate the loading of TOPBP1 to the DNA lesion. Will ERK phosphorylate ATR, ATRIP, or other components to facilitate ATR-ATRIP recruitment to RPA-coated ssDNA?

ERK activity enhances the repair of DSBs via HR (Golding et al., 2007), and inhibits NHEJ (Wei et al., 2013). These observations support that ERK plays a role in the choice of DSB repair pathways. As 53BP1 and BRCA1 are critical in the commitment to NHEJ and HR, respectively (Chapman et al., 2012), it will be of interest to see whether ERK modulates the formation of 53BP1 and BRCA1 nuclear foci in response to DSBs. Evidence suggests that ERK reduces DSB-induced activation of

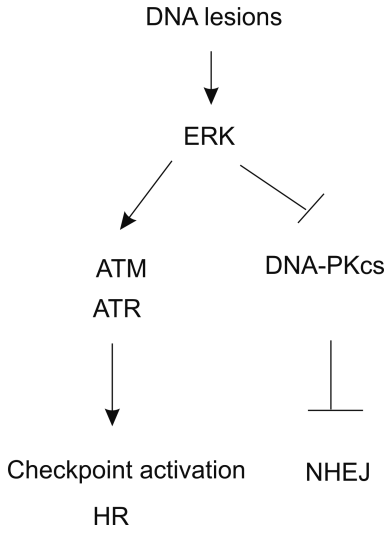

Fig. 1. Summary of ERK-modulated activation of PIKKs in DDR. DNA lesions induce ERK activation, which facilitates the activation of ATM and ATR. This enhances DNA damage-induced checkpoint activation and HR-mediated DSB repair. ERK activity can also inhibit DSB-induced DNA-PKcs activation and thus reduces NHEJ. It should be stressed that ERK may also facilitate NHEJ (see text for details).

DNA-PKcs (Wei et al., 2013). Activation of DNA-PKcs requires binding to the Ku70/80 heterodimer and DSBs (Gottlieb and Jackson, 1993; Suwa et al., 1994; Uematsu et al., 2007). Knockdown of either ERK1 or ERK2 was without effects on the formation of Ku70 nuclear foci but enhanced DNA-PKcs activation and the nuclear foci of S2056-phosphorylated DNA-PKcs (Wei et al., 2013), suggesting that ERK may inhibit the association of DNA-PKcs with Ku-associated DSBs. Alternatively, ERK kinases may phosphorylate DNA-PKcs, Ku, or other components, leading to inhibition of DSB-induced activation of DNA-PKcs.

Concluding remarks

Although the physiological relevance of ERK contributions to DDR and the detailed mechanisms governing ERK activation and ERK-facilitated activation of PIKKs will require future investigations, accumulating evidence clearly demonstrates that ERK kinases contribute to a variety of aspects of DDR, checkpoint activation and DSB repair. In view of the apical role of PIKKs in initiating DDR, the observations that ERK kinases regulate the proper activation of ATM, ATR, and DNA-PKcs (Fig. 1) further support the relevance and importance of ERK in DDR execution. Illustration of ERK-modulated activation of PIKKs will advance our understanding of DDR.

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