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REVIEW ARTICLE

PHOSPHATIDYLINOSITOL-3-KINASE RELATED KINASES (PIKKS) IN RADIATION-INDUCED DNA DAMAGE

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Summary

This review describes a drug target for cancer therapy, family of phosphatidylinositol-3 kinase related kinases (PIKKs), and it gives a comprehensive review of recent information. Besides general information about phosphatidylinositol-3 kinase superfamily, it characterizes a DNA-damage response pathway since it is monitored by PIKKs.

Key words: PIKKs; ATM; ATR; DNA-PK; Ionising radiation; DNA-repair

ABBREVIATIONS

DSB - double stand breaks,
IR - ionising radiation,
p53 - TP53 tumour suppressors,
PI - phosphatidylinositol.

INTRODUCTION

An efficient cancer treatment means to restore controlled tissue growth via interfering with cell signalling pathways regulating cell-cycle and apoptosis. Among many treatment strategies, targeted cancer therapy and radiation play a pivotal role. Since cancer is one of the leading causes of death worldwide, it is reasonable to invest time and resources in the enlightening of mechanisms, which underlie radio-resistance.

The aim of this review is to describe the family of phosphatidyinositol 3-kinases (PI3K) and its functional subgroup - phosphatidylinositol-3-kinase related kinases (PIKKs) and their relation to repairing of radiation-induced DNA damage. Besides PI3K classification, we give a detailed description of the mechanisms of activation as well as their downstream substrates. Taken together, this paper concerns DNA damage repair induced by gamma-radiation and focuses on the role of PIKKs.

Phosphatidylnositol 3-kinase family

Protein kinases are generally believed to be an effective drug target. Many studies and clinical trials have been focused on inhibition of Epidermal

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Growth Factor Receptor family (EGFR), Breakpoint cluster region - Abelson murine leukemia viral oncogene homolog 1 fusion protein (Bcr-Abl) or Platelet-Derived Growth Factor Receptors (PDFGR) kinases but recently PI3K have come under the spotlight [1][2][3].

PI3K were originally described by the group of Lewis Cantley. They were the first ones who observed a close correlation between phosphoinositide (PI) kinase activity and transforming ability of viral oncoproteins [4]. They also reported unique substrate specifity towards PI and that PI3K phosphorylates phosphatidyinositol bisphosphate (PIP2) on the position 3 of the inositol ring, thus producing phosphatidylinositol (3,4,5)-trisphosphate (PIP3) [5].

PI3K classification

PI3K superfamily is used to be generally classified into three classes: I, II, and III. The classification is based on primary structure; catalytic and adaptor/regulatory subunits associate into the heterodimers, thereby affecting PI3K response to a wide variety of stimuli. Another criterion is substrate specifity since each PI3K class produces characteristic lipid second messengers. In this paper, we describe in detail an additional group of more distantly related and structurally heterogeneous enzymes functioning as protein serine/threonine kinases, which are involved in monitoring of genomic integrity and control signalling in order to regulate cell growth. These kinases will be referred to as class IV (see Table 1.).

Table 1. Overview of members of phosphatidylnositol 3-kinase family, their substrates and functions.

Class	Catalytic / Regulatory subunit		Substrate	Function	Reference
IA	p110α, p110β, p110δ	p85α, p55α, p50α, p85β, p55γ	phosphoinositide(4,5) bisphosphate (PIP2)	cell growth, proliferation, survival, glucose homeostasis, metabolism	[7] [81]
IB	p110γ	p101, p84		immune and inflammatory processes	
II	C2α, C2β, C2γ	---	phosphatidylinositol (PI) and PI(4)P, PI(4,5)P2	membrane trafficking	
III	Vps34	p150	PI	autophagy	
IV	**ATM**		e.g. ATM, H2A.X, p53, chk-2, mdm2, BRCA1, Nbs1, Mre11, Phas-I, etc.	DNA repair, cell cycle progression, apoptosis	[12] [33] [82]
PIKKs	**ATR**		p53, Mre11, chk-1, BRCA2, DNA pol-δ, RPA, Phas-I	replication block, ssDNA repair	[82]
	DNA-PK		DNA-PK, XRCC4, Ku70/80, XLF, Artemis, DNA lig IV, H2A.X, p53, Rad17, BRCA1, 123F-2	non-homologous end joining	[42] [82]
	mTOR		$p70^{s6k}$, 4E-BP1, Akt/PKB	cell growth, metabolism and survival, protein synthesis, and transcription	[64]
	SMG-1		p53	nonsense-mediated mRNA decay	[78]
	TRRAP		no kinase activity	embryonic development, cell cycle progression and mitotic control	[77]

PI3K Class I

This is the most studied subgroup due to its significance in human cancer. Class I PI3Ks are further divided into IA and IB subset based on sequence similarity. IA subset consists of heterodimers comprising a catalytic (p110) and regulatory subunit (p85). Three *PIK3R* genes give rise to five p85 isoforms (*PIK3R1* for p85α, p55α, p50α; *PIK3R2* for p85β; and *PIK3R* for p55γ) as

a consequence of splice variants. *PIK3CA*, *PIK3CB*, and *PIK3CD* produce isoforms of catalytic subunit p110α, p110β, and p110δ. Each p85 isoform can associate with any of p110 isoforms. IB subset consists of heterodimers between p110γ (similarly to other p110s) and a distinct regulatory subunit (p101 or p84). Both subunits are encoded by a single gene [6]. More specifically, p110α and p110β are both expressed in all cells and they affect cellular proliferation or insulin signalling, respectively. On the contrary, p110γ and p110δ are primarily expressed in leukocytes. Thus, they are involved in immune and inflammatory processes. Importantly, p110α is widely mutated or amplified in human cancer [7].

The preferred substrate of class I, PI3-kinases is phosphoinositide(4,5)bisphosphate (PIP2). This is also a substrate for members of the PI-phospholipase C family and the product of phosphatase and tensin homolog (PTEN; a tumour suppressor) dephosphorylation of PI(3,4,5)P3. Phosphorylation of PIP2 by PI3K generates PI(3,4,5)P3. PI(3,4,5)P3 and its 5'-dephosphorylation product, PI(3,4)P2, are important second messengers that coordinate and promote cell survival, growth, protein synthesis, mitosis, and motility. PI(3,4)P2 is also produced by Class II PI3K from PI(4)P. PtdIns(3,4,5)P3 produced by PI3-kinase is also involved in cell motility via regulation of Rho-GTPases, RhoA, Rac-1, and Cdc42 ([7]). Cell survival, mitosis, and protein synthesis are promoted by PI3-kinase-dependent activation of the PDK/AKT(PKB) pathway. Besides that Class I PI3Ks are involved in proliferation, glucose homeostasis, and metabolism [8].

PI3K Class II and III

Class II molecules are, unlike class I and III PI3Ks, monomers comprising three catalatylic isoforms (C2α, C2β, and C2γ) without regulatory subunits. C2α and C2β are expressed in all cells but C2γ is expressed only in hepatocytes. Class II PI3Ks are involved in membrane trafficking. Class III kinases are more similar to Class I, since they are composed of a regulatory subunit (p150) and a catalytic subunit (Vps34). They function in regulation of autophagy and trafficking proteins and vesicles [9].

Class II PI3-Ks preferentially phosphorylate PI and PI(4)P to form PI(3)P and PI(3,4)P2, respectively. Class II PI3-Ks also phosphorylate PI(4,5)P2 in the presence of phosphatidylserine (PS). Class III PI3-Ks preferentially phosphorylate PI to form PI(3)P, which has important roles in vesicular and protein trafficking. In addition, Class III PI3Ks are involved in targeting lysosomal enzymes to the endocytic pathway [7].

PI3K Class IV alias PIKKs

Class IV PI3Ks are known as phosphatidylinositol-3 kinase-related kinases (PIKKs). This class comprises of ataxia telangiectasia mutated kinase (ATM), ataxia telangiectasia and Rad3 related kinase (ATR), DNA-dependent protein kinase (DNA-PK), and mammalian target-of-rapamycin (mTOR). These members of the PI3K superfamily are protein serine/threonine kinases, which are involved in processes of tumour diseases development and function in signalling pathway called DNA-damage response (DDR). In this paper, we will focus on the individual members of class IV, since they are linked to DNA repair. ATM and DNA-PK respond mainly to double strand breaks (DSB), whereas ATR is activated by single-stranded DNA and stalled DNA replication forks. In all cases, activation involves their recruitment to the sites of damage [10]. In the next sections, we describe the individual members of class IV and their participation in DDR. Finally, we mention two other members of the family: suppressor with a morphological effect on genitalia family member (SMG-1), and transactivation/transformation-domain-associated protein (TRRAP).

Ataxia telangiectasia mutated kinase (ATM)

Activation of ATM is one of the first steps linked to DNA damage response after the exposure to ionising radiation [11], [12]. It is triggered by formation of the most severe forms of DNA lesions - DSB. Undoubtedly, ATM is indispensable in regards to DSB reparation, since it is involved in DNA repair and regulates all three cell-cycle checkpoints and apoptosis [13]. During ATM activation after irradiation, the key factor is a rapid intra-molecular phosphorylation at serine 1981, which induces dissociation of an inactive dimer and triggers ATM activity [14]. Also a specific protein complex is required for its activation consisting of Mre11, Rad50, and Nbs1 protein (MRN) [15]. It was proved that ATM is not activated without MRN complex and that mutation of its components leads to a genetic disorder as neurological abnormalities, radio-sensitivity, cell cycle defects, genomic instability, and cancer predispositions [16]. MRN complex is

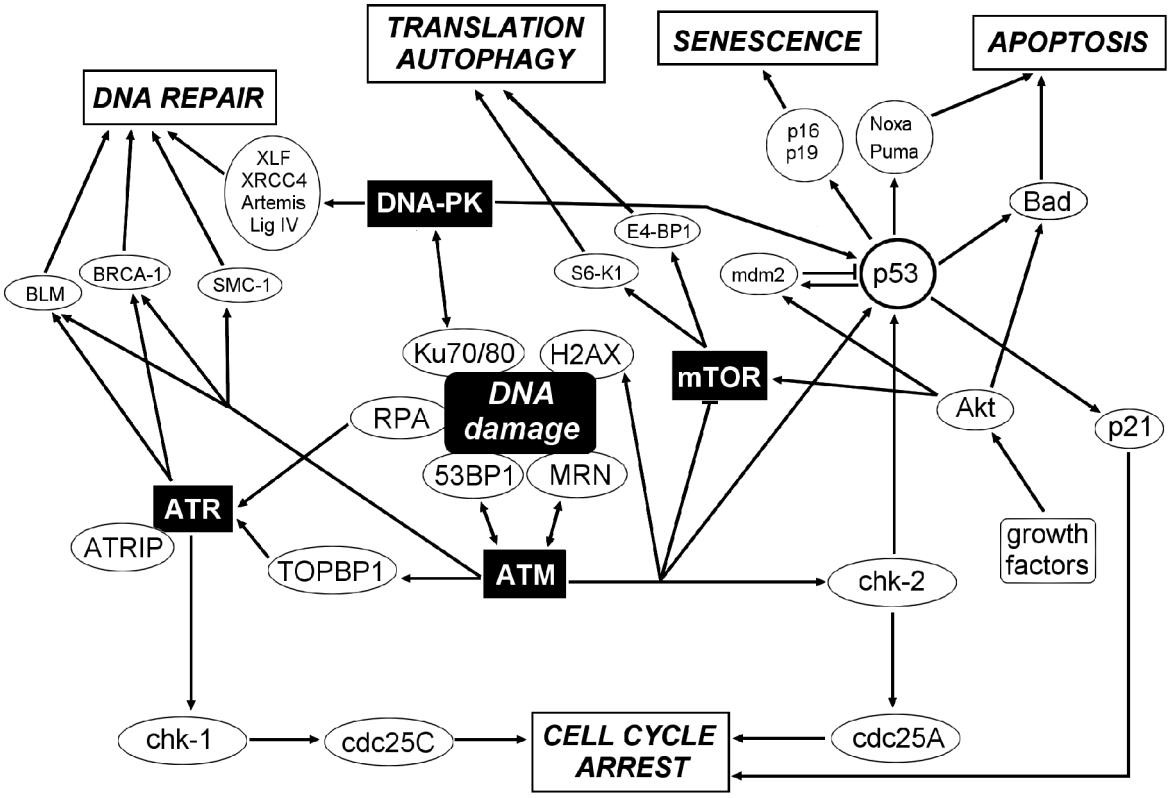

Figure 1. IR induces molecular mechanisms, which can lead either to cell cycle arrest (ATM/ATR) in order to provide cells enough time to repair DNA damage (DNA-PK) or to programmed cell death or senescence. Signalling via mTOR initiates translation and regulates autophagy and cell nutrition.

associated with chromatin during DNA replication and it can recognize DSB and transmit this information to ATM by attraction of ATM to the damaged DNA [17].

Importantly, MRN complex is able to bind DNA without involvement of active ATM suggesting that MRN complex is the entire sensor of DSB [18]. Anyway, once activated, ATM is the central DSB signalling transducer. Falck and colleagues ([10]) reported an interesting finding that Nbs1 is dispensable for ATM activation, but its C-terminal motif is required for ATM localization in the site of damage. Another protein of MRN complex, Rad50, functions as a protective chromosomal factor. It impedes excessively rapid shortening of telomeres and so-called end-to-end joining of sister chromatids [19]. The particular proteins of MRN complex regulate each other. Nbs1 recruits Mre11 into the nucleus while Mre11 increases Nbs1 stability and Mre11 exhibits specific endonuclease activity towards DNA with DSB while Rad50 inhibits this feature [18], [20].

Upon gamma-irradiation, ATM associates with chromatin and also with histonedeacetylases, thus facilitating access of homologous recombination (HR) proteins to the sites of damaged DNA [13]. The very early step in the DNA damage response is phosphorylation of histone subtype H2A, class H2A.X. This process can be executed by two mutually independent protein kinases – DNA-PK and ATM – and therefore it might be observed even in ataxia telangiectasia cell lines [21]. H2A.X phosphorylated on serine 139 (referred as γH2A.X) can be visualized by a suitable antibody via immunofluorescence as a discrete spot (focus) and it has been reported that it is localized in the area up to 2×10^6 basis from the site of DSB [22]. This might be also exploited in biodosimetry, since the formation of ionising radiation-induced foci seems to be dose-dependent [23]. Although γH2A.X is not essential for non-homologous end-joining (NHEJ) and HR, it seems to be an important modulator of both [24]. γH2A.X functions as a protein docking site and it is likely that it is needed for retention of some proteins

participating on DNA repair rather than binding them and is crucial for assembly of reparation complex in the site of DSB [25].

In the last two decades, a large number of ATM substrates, which are activated by phosphorylation, were identified, but among these tumour suppressor protein p53 (TP53) is outstanding, because a wide range of studies links its activation to the process of DNA repair [26]. Furthermore, it is a key mediator of the cell fate, since it is capable of initiation of cell-cycle arrest, senescence, or apoptosis via activation of p53-inducible genes [27], [28]. In a normal cell, tumour suppressor p53 is present in a latent form with low affinity to specific sequences of DNA, but after genotoxic stress its activity increases substantially. Regulation of p53 activity after exposure to IR (not UV-radiation) is to a great extent ATM-dependent and can be controlled via subcellular localization, proteolytic degradation mediated by ubiquitin, or by allosteric modification on the main DNA binding domain [29]. In order to transfer p53 to cytoplasm, ATM phosphorylates murine-double-minute protein-2 (mdm2), which is an E3 ubiquitin ligase [30] essential for targeting and effective p53 degradation in an auto-regulatory bond.

Protein p53 induces mdm2 transcription, which directly binds to p53 N-terminus, thus blocks its further transcriptional activity and maintains p53 degradation ([31]). If the cell is exposed to the DSB-inducing stress such as IR, this tight auto-regulatory bond is interrupted and p53 is phosphorylated in order to make p53 more resistant to the inhibitory effects of mdm2. Moreover, p53 transcriptional activity is stimulated. P53-mdm2 model is controlled by ATM directly via p53 phosphorylation on serine 15 and indirectly via phosphorylation on serine 20 that is conducted by checkpoint kinase-2 (chk-2), a kinase activated by ATM [32]. These phosphorylations are rapid, detectable very soon after irradiation [33] and they make p53 more resistant to the inhibitory effects of mdm2. Our group proposed phosphorylation on serine 15 as a potential biodosimetric marker [34].

Among the plethora of p53 transcriptional targets, one is outstanding in the terms of regulation of G1/S checkpoint. It is p21 protein (WAF1/Cip/Sdi1), which together with p27 and p57 creates a family of proteins sharing the ability to induce the cell-cycle arrest via inhibition of a wide range of cyclin-dependent kinases (cdk; [35]). While regulating G1 checkpoint, ATM controls also the entry into S-phase, which undermines phenotype of so-called radio-resistant DNA synthesis. Falck et al. [36] showed that impairment in ATM/chk-2/Cdc25A/cdk2 checkpoint pathway results in uncontrolled cellular inhibition of the DNA synthesis. The same group also proved that a parallel mechanisms, by which ATM controls S-checkpoint, exists - demanding MRN complex [37].

Finally, ATM regulates G2/M checkpoint, necessary for the cell cycle arrest of the cells, which were irradiated in the G2-phase and need to repair eventual DNA damage. Processes in this checkpoint are checkpoint kinase-1 and checkpoint kinase-2-dependent. Checkpoint kinases (activated via ATM/ATR) subsequently posses ability to inhibit activation of Cdc25C phosphatase required for activation of further proteins (cyclin B1 and cdk1) and for progression of the cell-cycle [38]. Thus ATM together with ATR regulates a wide range of target molecules by phosphorylation.

DNA-dependent protein kinase (DNA-PK)

DNA-PK is a serine/threonine kinase composed of a 460 kDa catalytic subunit (DNA-PKcs) and a DNA-binding het¬erodimer consisting of two subunits: Ku70 (70 kDa) and Ku86 (86 kDa), which is sometimes referred to as Ku80 (reviewed in [39]). The importance of DNA-PK derives from its role in non-homologous end joining (NHEJ). NHEJ is considered to be the major DSB repair pathway. The mechanisms of NHEJ and also HR are well covered in detail in a recent review of Kasparek and Humphrey [40].

Heterodimer of Ku70/80 ensures initiation of NHEJ and it binds to double-stranded DNA broken ends before DNA-PKcs binds and is activated [41]. There was another helping protein discovered in highly radio-sensitive cell lines with defective DSB reparation, which was named X-ray cross complementing protein (XRCC4). Matsumoto et al. [42] reported that it is specifically phosphorylated by DNA-PK in IR-irradiated cells. XRCC4 has been shown to bind to an important part of the system - DNA ligase IV [43]. In mice lacking XRCC4 or DNA ligase IV gene, massive apoptosis occurs in embryonic neural cells [44] and mutations in human fibroblast cell line 180BR (derived from patient with lymphatic leukaemia) leading to higher radio-sensitivity, were according to Riballo et al. [45] linked to DNA ligase IV and the inability to repair the radiation damage by NHEJ.

Bogue et al. [46], proposed relation of DNA-PKcs with DNA repair and genomic stability, since they observed extreme radio-sensitivity of the cells lacking DNA-PKcs. The same group reported that gamma-irradiated DNA-PKcs$^{-/-}$ mice remain viable, but immunodeficient and it seems that some aspects of the DNA-PKcs function are unique and mutations of DNA-PKcs or Ku in humans have lethal consequences.

Recently, Van der Burg et al. [47] have identified the first human DNA-PKcs gene mutation in immunodeficient patient with only mild radiosensitive. The mutation did not result in the loss of enzymatic activity or deficient autophosphorylation of DNA-PKcs, but affected activity of Artemis, a kinase required for nucleolytic processing of DNA ends.

Activated DNA-PKcs phosphorylates a number of proteins *in vitro*, including p53, transcription factors, RNA polymerase, Ku70/Ku80, XRCC4-like factor (XLF), Artemis, DNA ligase IV [48]. Besides that, DNA-PKcs autophosphorylation was reported at multiple sites, including threonine 2609, which results in a loss of DNA-PK kinase activity [49]. Hammel et al. [50] have shown that site-specific auto-phosphorylation induces a large conformational change that opens DNA-PKcs and promotes its release from DNA ends. Additionally, it seems that Ku and DNA-PKcs play a pivotal role in other cellular processes, like telomere maintenance, transcription of specific genes or promotion of apoptosis [51].

ATM-Rad3 related kinase (ATR)

The third one of the group of enzymes that is primarily responsible for signalling of the presence of DNA damage is ATM and Rad3-related kinase (ATR). Activation of ATM and DNA-PKcs is triggered by DSB, while ATR responds to replication blocks or other conditions that result in formation of single stranded DNA gaps (ssDNA; reviewed in [52]). ATR seems to be the most versatile PIKK DNA-damage-responsive kinase, since it is activated not only by IR, but also by UV-radiation, methyl methansulfonate and cis-platinum, and many inhibitors of replication such as hydroxyurea and aphidicolin [53]. Likewise ATM is recruited to DSB indirectly via MRN complex ([16]), or DNA-PK is recruited by Ku70/80 [41], Cortez et al. identified an ATR-interacting protein (ATRIP) that is phosphorylated by ATR [54]. ATRIP is an essential component of the ATR-dependent damage checkpoint pathway, since it binds to Replication protein A (RPA), which coats most forms of ssDNA in the cell. Additionally, RPA-coated ssDNA is sufficient to recruit the ATR-ATRIP complex, but it is not sufficient for ATR activation [55]. The critical activator of ATR is TOPBP1 (DNA topoisomerase II-binding protein 1) [56]. TOPBP1 contains an ATR activation domain and it was shown to induce a large increase in the kinase activity of human ATR [57].

ATR is indispensable in replicating cells perhaps due to the ubiquitous presence of DNA lesions and replication stress and its primary function is to regulate progression of cell cycle into G2-phase [58]. ATR is well-known for phosphorylation of chk-1 but it activates also other proteins involved in recombination, such as breast cancer 1 (Brca1), Werner syndrome protein (WRN), and Bloom's syndrome protein (BLM) [59]. Prevo et al. published a study on pancreatic cancer cells where a specific ATR inhibitor VE-821 inhibited chk-1 phosphorylation and increased radio-sensitivity via shortening G2/M cell cycle arrest and inhibition of homologous recombination [60]. While ATR phosphorylation of chk-1 helps to spread the damage signal, many of the critical functions of ATR are associated with chromatin and more specifically with promoting replication fork stability and recovery of stalled forks to ensure completion of replication. Additional ATR substrates include the replication factor C complex, RPA1 and RPA2, the minichromosome maintenance protein complex (MCM2-7), MCM10, and several DNA polymerases [59].

Most ATR substrates can also be phosphorylated by ATM, and the major functions of ATR and ATM in cell cycle control are overlapping but non-redundant. Crosstalk between these pathways often occurs as a consequence of inter-conversion of activating DNA lesions. For instance, in irradiated hypoxic cancer cells Pires et al. reported that a part of a large DNA damage and decrease in Hypoxia-inducible factor 1, ATR inhibition by VE-821 induces phosphorylation of histone H2AX, a phenomenon well-described for ATM or DNA-PK [61]. Moreover, although ATR primarily responds to replication stress, it is also activated by presence of DSB. It was proved that ATM is capable of activating ATR through phosphorylation of TOPBP1 [62].

In the past decade, ATM and ATR pathways were thought to act in parallel but nowadays due to accumulating evidence it has become apparent that

their inter-connection is much more complex. What is then the reason for rapid lethality at the earliest embryonic stages in cells with defective ATR-chk-1 pathway, when cells with mutations in ATM (or other components required for HR, such as BRCA1 and BRCA2), can survive even at the cost of genomic instability and cancer predisposition [63]? ATM used to be often described as the initiator of the checkpoint response and ATR was characterized as the kinase that maintains it. Nowadays, we rather think of ATM and ATR as partners in the DSB response.

mTOR

In mammalian cells, there are three other PIKKs: transactivation/transformation-domain-associated protein (TRRAP), mammalian target of rapamycin (mTOR), and suppressor with morphological effect on genitalia family member (SMG-1).

mTOR integrates responses from a wide variety of signals such as nutrients (amino acids, glucose), hormones (insulin), growth factors and cellular stresses to regulate cell growth, metabolism, and survival, protein synthesis, and transcription [64]. The kinase consists of two complexes mTORC1 and mTORC2; while the former one is inhibited by a bacterial product rapamycin, the latter one is rapamycin-insensitive. Although a precise mechanism of activation of mTOR is not fully understood, PI3K and protein kinase B (Akt) seem to be the key modulatory factors [65]. The PI3K/Akt signal transduction pathway A is a principal pathway that signals through mTOR and it is critically involved in the mentioned mediation of cell survival and proliferation, mainly by anti-apoptotic Akt-dependent phosphorylation of Bad [66].

The activation of mTOR enhances protein translation via phosphorylation of eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and S6 kinase (S6K1), which are the main targets of mTORC1 [67]. S6 phosphorylation has been used as a biomarker for mTOR activation [68]. mTORC2 has been shown to function as an important regulator of the cytoskeleton [69]. Since mTOR is inhibited by rapamycin, a new line of anticancer drugs, such as CCI-779 and RAD001, emerged. These substances exhibit significant anticancer activity in various tumour cell lines and lead to inactivation of ribosomal S6K1 and inhibition of 4E-BP1 resulting into accumulation of cells in the G1 and potential apoptosis [70]. In the terms of sensitivity towards IR, rapamycin (known as sirolimus) and temsirolimus were recently shown as selective effectors of the radiation therapy response, although dependent on relative cell cycle kinetics [71]. Interestingly, Le Guezennec et al. reported that Wip1 phosphatase (a known negative regulator of ATM-dependent signalling) regulates autophagy, obesity, and atherosclerosis via inhibition of mTOR, thus indicating existence of a non-canonical ATM-mTOR signalling pathway [72]. Consistently, mTOR inhibitors radiosensitize cells via disruption of the major DNA repair pathways. As it was proved by Chen et al., treating irradiated MCF7 breast cancer cells with rapamycin results in impaired recruitment of BRCA1 and Rad51 to DNA repair foci (both essential for HR) and they reported a significant suppression of HR and NHEJ [73].

Recently, a surprising link between mTOR and DSB repair was pointed out by Robert et al. [74]. Their study provides the evidence of acetylation-regulated degradation of Sae2 (a protein that negatively regulates DNA damage checkpoint signalling) by autophagy. Robert et al. induced hyperacetylation of proteins by inhibition of histone deacetylase activity. Autophagy is executed by proteins, which respond to signals from the mTOR, thus its inhibition with rapamycin triggers autophagy in irradiated cells resulting in decreased level of Sae2 and subsequent failure to repair DSB [75]

SMG-1 and TRRAP

TRRAP retains most of the catalytic domain but lacks the residues that are essential for binding ATP and it is the only PIKK member, which does not possess the kinase activity towards Ser or Thr residues. Nevertheless, it has been found to have an essential role in embryonic development, cell-cycle progression and mitotic control [76].

SMG-1 is the sixth and the newest member of the mammalian PIKK family. It plays a critical role in the mRNA quality control system termed nonsense-mediated mRNA decay (NMD) and protects the cells from the accumulation of aberrant mRNAs.

The complete and detailed characterization of mTOR, SMG-1, and TRAPP is far beyond the extent of this paper. For further reading regarding these kinases we recommend several other reviews (e.g. [65], [77], [78], [79] and [80]).

CONCLUSION

Taken together, the whole kinase family exhibits functional heterogeneity. Despite that some of its members co-operate together while they orchestrate DNA damage response. Our knowledge about the DNA damage signalling pathway has greatly increased over the past several years. However, new questions about the sensors and transducers, which mediate the DNA damage response, are still arising, especially when proteomic analysis have identified hundreds of potential substrates of ATM, ATR, and DNA-PK. A need to understand the mechanisms of their action is driven by the fact that they are involved in the processes which underlie radiation resistance. Nowadays, we are expecting with interest the results of clinical studies focused on inhibition of DNA repair in cancer cells either by small inhibitor molecules or small interfering RNA (siRNA), because development of efficient therapeutic tools and deeper comprehension of DNA damage response will establish new platforms for treatment strategies in oncology.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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