Journal of Chemical, Environmental and Biological Engineering
2018; 2(2): 60-69
http://www.sciencepublishinggroup.com/j/jcebe
doi: 10.11648/j.jcebe.20180202.14
ISSN: 2640-267X (Print); ISSN: 2640-2645 (Online)

Review Article

Investigation of Novel Functions of KICSTOR Components in the DNA Damage Response

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To cite this article:
Manideep Chowdary Pachva, Alexander Ryan. Investigation of Novel Functions of KICSTOR Components in the DNA Damage Response. *Journal of Chemical, Environmental and Biological Engineering*. Vol. 2, No. 2, 2018, pp. 60-69. doi: 10.11648/j.jcebe.20180202.14

Received: October 7, 2018; **Accepted**: November 10, 2018; **Published**: December 26, 2018

Abstract: The mechanistic target of rapamycin complex (mTOR) is an atypical serine/threonine kinase which acts as a global cellular regulator of growth and cell survival in response to environmental cues and is a member of the phosphoinositide 3-kinase (PI3K)-related kinase family. Through the numerous inhibitions and initiations of catabolic and anabolic processes respectively, mTORC1 is also a major promoter for cell-cycle progression. mTORC1 can be activated by growth factors such as insulin as a downstream target of PI3K signaling. It is expressed in all somatic cell types plays vital roles in axonal movement, neuronal plasticity and development in the brain. The aberrant activation of mTORC1 has been implicated as one of the leading causes of Tuberous Sclerosis and Focal Epilepsies. Constitutively active mutations in mTOR complex subunits and their upstream signalling proteins have also been documented in over 30% of Cancers, such as the recently recognised links to prostate and colon cancer. Some PI3K/mTOR inhibitors have also been shown to potently inhibit DNA damage responses in non-small cell lung cancer (NSCLC) cell lines suggesting mTOR plays a key role in DNA damage response (DDR) mechanisms. This review focuses on delineating the mTOR pathway, mechanism of mTOR inhibitors and their possible role in inhibiting the DDR mechanism.

Keywords: mTOR, Cancer, DDR, KICSTOR

1. Introduction

The Hallmarks of Cancer

Cancer cells exhibit specific “hallmarks” including alterations to multiple aspects of cell function, such as unlimited proliferation. These “hallmarks” have become a useful model in the determination of what defines a cell as cancerous. These hallmarks include resistance to cell death, replicative immortality, tumour- supporting angiogenesis, invasion and metastasis, metabolic reprogramming, evasion of immune destruction, bypassing growth suppressors and the presence of sustained proliferative signalling. Sustaining proliferative signals refers to a cancer cells ability to sustain chronic proliferation and could is arguably the most fundamental trait of these cells [1]. The acquisition of sustained proliferative capabilities can be achieved through overproduction of growth factors that bind to their cognate receptors in an autocrine manner, overproduction of signals which stimulate stromal cells to produce various growth factors, or overexpression of ligand receptors that lead to hypersensitivity in constitutively low concentrations of growth factors, contributing to sustained proliferative capabilities [1-2]. Sustained proliferation may occur through mutations in the pathway components resulting in a constitutively active state that constantly stimulates downstream effectors. These mutations do not necessarily have to occur at the level of the receptor but can also arise downstream of this, affecting the negative feedback loops that often limit signal transduction [1]. An interesting example of how the inhibition of a protein involved in cell growth and survival can lead to upstream feedback and subsequent over activation of upstream signalling pathways is the mechanistic target of rapamycin (mTOR) Kinase – or more specifically mTOR complex 1 (of which there are 2

known complexes; mTORC1 and mTORC2). Due to mTOR kinase's negative feedback loop with the protein Phosphoinositide 3 Kinase (PI3-K), it's overstimulation leads to subsequent inhibition of its upstream effectors. mTORC1 activation by growth factor receptor (GFR) activates PI3- K and leads to a large number of downstream effects including cell growth and proliferation. However, as demonstrated experimentally, subsequent inhibition of mTOR in cancer cells by Rapamycin leads to an overactivation of PI3- K and therefore PKB/Akt pathway. This shows how the facets of mutation are interlinked with the overall cell signalling cascade and that both over- or under- expression of a gene can lead to abnormal cellular growth and proliferation [3].

Alongside accumulation of mutations within a single cell, the cellular environment has been shown to play a key role in cancer progression; A prime example of this is with the crypts of the colon and intestines. In a normal state, Wnt signalling drives differentiation of progeny cells from their Intestinal Stem Cells (ISCs) and maintains the homeostasis of these stem cells. The high concentration of the Wnt molecule at the base of these crypts creates an ISC niche which allows the continuous propagation of these cells, but differentiation into all cell types of the tissue is driven by the decrease in Wnt signalling as progeny cells migrate up the crypt after each mitotic event. Therefore, overexpression of Wnt signalling has been implicated as a major driver in both tumour growth and metastasis of Colorectal Carcinomas (CRCs). This disruption of environmental cues shows how a careful interplay between individual somatic mutations and external signals can drive tumourigenesis.

2. DNA Damage and Repair Mechanisms

All organisms have a biological imperative to preserve and transmit their genetic material faithfully across generations. Cells are constantly subject to DNA damage from a variety of exogenous and endogenous genotoxic sources, experiencing as many as 10^5 DNA lesions a day [4-5]. Endogenous sources can cause alkylation, oxidation or hydrolysis of DNA by free radical oxygen species or through in appropriate deoxyribonucleotide placement during replication [6]; whereas environmental sources of DNA damage include UV light, Ionising radiation, cigarette smoke inhalation or chemotherapeutic drugs. These insults result in very different forms of DNA damage, including cyclobutane pyrimidine dimers (CPDs), single strand and double strand breaks or intra-strand crosslinks [7]. To maintain genomic viability, organisms have evolved distinct specific DNA repair mechanisms to deal with each of the various forms of DNA damage. Figure 2 contains a list of various repair mechanisms at work in a mammalian cell [8]. As previously described, the ability of the cell to respond to and repair DNA damage through these various mechanisms is a major determinant of its ability to preserve homeostasis and alterations in these pathways can lead to predisposition to cancer. This is exemplified by the occurrence of cancer predisposition syndromes such as Fanconi Anaemia which is associated with a germline defect in DNA repair genes [8-9]. Genome instability is an important factor in cases of both inherited and sporadic tumourigenesis; for example, mutations in BRCA1 and BRCA2 are predispositions to breast cancer and ovarian cancer. Many cases of inherited cancer are caused by genome instability resulting from an underlying defect in DNA repair, and in these cancers the 8 tenets (see figure 1) and predominantly driven by DNA damage and mutation through genome instability which leads to aberrant cell proliferation and carcinogenesis through a multistep process [1].

Specific DNA repair mechanisms exist to deal with specific forms of DNA damage. Miss- paired DNA bases formed during replication are repaired via Mismatch repair (MMR) mechanisms but if small erroneous base pair alterations have occurred due to chemicals then base excision repair (BER) mechanisms are activated [6, 8]. Pyrimidine dimers formed following exposure to UV radiation are repaired via the action of nucleotide excision repair (NER) mechanisms, which recognise double helix distortions in the DNA. This can be subcategorised into global genome NER and transcription coupled NER [10]. Double strand breaks are usually identified as the most dangerous form of DNA lesion and several repair mechanisms compete depending on the cell cycle phase, type of damage and the epigenetic modification of nearby histones; in S and G2 phases error-free homologous recombination (HR) can occur, where by the sister chromatid of the diploid cell is used as a template for repair. In all phases of the cell cycle, non-homologous end joining (NHEJ) occurs, where religation of the DNA strands are highly prone to inaccuracies and can prove tumourigenic [8, 11]. The importance of these repair mechanisms can be seen with rare recessive diseases such as XP in which mutations in the proteins involved in NER are mutated and leads to highly sensitive skin to UV light because most UV damage to DNA is in the form of helical distortions and are repaired by NER under normal circumstances. This ultimately leads to a much higher risk of developing skin carcinomas (>1000 fold) but also an increase risk in other malignancies such brain, lung and blood cancers (~20 fold) before the age of 20 [4].

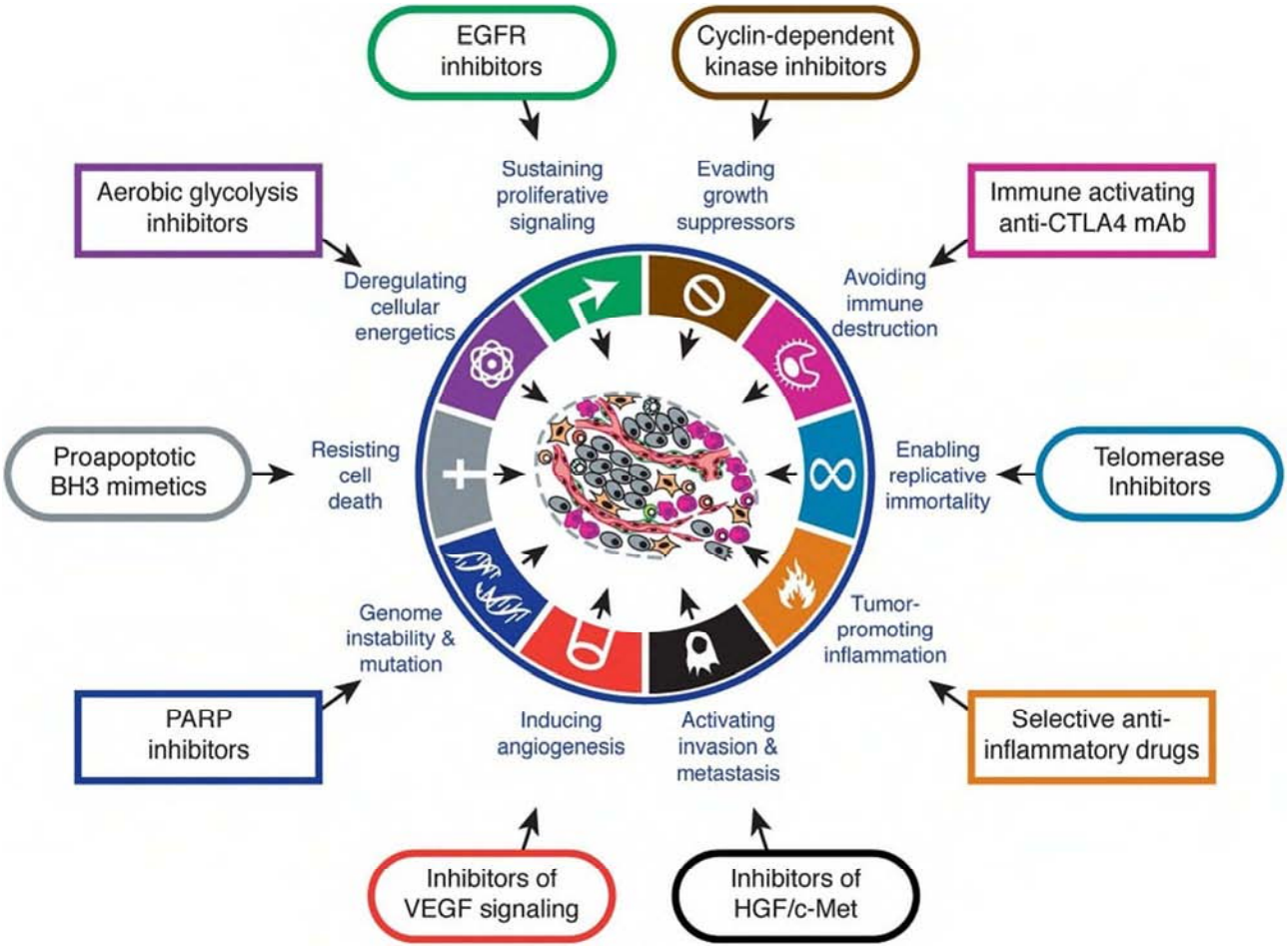

Figure 1. *A diagram which summarises the 8 basic "hallmarks" of cancer. These are: Enabling Replicative immortality, inducing angiogenesis, sustaining proliferative signalling, activating invasion and metastasis, evading growth factors, avoiding immune destruction and resisting cell death. Underlying these hallmarks is genome instability and tumour promoting inflammation. At each point, an outline of therapies which tackle each aspect. These hallmarks each define specific pathways which are found to be mutated in many cancers, such as the tumour suppressor p53 mutation (adapted from [1]).*

Distinct DNA repair systems are specialized to repair the various types of DNA lesions.

Repair mechanism	Lesion feature	Genotoxic source (examples)
Base excision repair (BER)	Oxidative lesions	Reactive oxygen species (ROS)
Nucleotide excision repair (NER)	Helix-distorting lesions	UV radiation
Translesion synthesis	Various lesions	Various sources
Mismatch repair (MMR)	Replication errors	Replication
Single stand break repair (SSBR)	Single strand breaks	Ionizing radiation, ROS
Homologous recombination (HR)	Double-strand breaks	Ionizing radiation, ROS
Non-homologous end joining (NHEJ)	Double-strand breaks	Ionizing radiation, ROS
DNA interstrand crosslink repair pathway	Interstrand crosslinks	Chemotherapy

Figure 2. *Outlining the type of DNA damage and what the causative agents involved in this type of damage are, alongside cellular repair mechanisms available in a mammalian cell depending on the type of damage (Image adapted from [4]).*

When a double strand break (DSB) occurs, ATM mediated phosphorylation of histone H2AX occurs quickly near the foci of the break and has been shown to be the first step in the recruitment and localisation of DNA repair proteins. This γ-H2AX foci formation occurs in a 1:1 manner with DSBs and immunofluorescence can be a useful tool in detecting

DNA damage in the context of cancer therapies and treatments [12]. DSBs can be repaired in 4 known pathways, HR, NHEJ, alternative-NHEJ and single strand annealing (SSA). Below figure 3 depicts the pathways that facilitate DSBs. For HR, PARP1 is recruited and enlists the proteins ATM & MRN to the site of damage along with TIP60 to stimulate ATM's kinase activity. ATM phosphorylation of p53 and CHK2 and other DDR factors resulting in the recruitment of MDC1 and other subsequent protein in a γ-H2AX dependant cascade, including BRCA1 to promote HR in S and G2 phase of the cell cycle. The complex which BRCA1 and the other proteins (MRN and CtlP) also promotes resection of the double strand break when NHEJ fails and the DNA ends become deprotected. EXO1 and BLM then promotes extensive DBS resection and the formation of RPA which coats 3' ssDNA ends. Following this, BRCA2 mediates the assembly of the protein RAD51 and subsequent strand invasion into the homologous DNA sequences. This recruitment of RAD51 is also regulated by the ATR pathway. After strand invasion, one of several eventualities will occur; EME1/MUS81 will cleave D loop structures formed and subsequently produce a crossover event, or RTEL1 will displace the strands and produce a non-cross over event. Alternatively, Holliday Junctions can be terminated by the protein complex – BLM/TOPOIII or resolved by GEN1 and SLX1/4 nucleases to produce crossover and non-crossover events respectively [8]. Alternatively, NHEJ is first initiated by Ku heterodimer (Ku70/Ku80) which binds in competition with PARP, to DNA break points and recruits' DNA-PKcs as well as other proteins to the site of damage to stabilise and prevent end resection through phosphorylation. Autophosphorylation of DNA-PKcs at T2609 cluster provides access to ARTEMIS and other end processing enzymes. Autophosphorylation of DNA-PKcs also mediates the prevention of over-processing by these enzymes through its five residue PQR cluster [8, 13]. Initial phosphorylation of DNA-PKcs can also be facilitated by ATM for regulation of HR when NHEJ does not work as well as a role in around 10% of NHEJ [14-15]. As a key pathway in multiple facets of cell survival, synthesis, anti-apoptosis and cell cycle progression and as the basis of this research project, focus will be placed particularly on the mTOR-Kinase pathway and mTORC1 with its role in DNA repair and pathology.

3. The Mechanistic Target of Rapamycin

The mechanistic target or rapamycin complex (mTOR) is an atypical serine/threonine kinase which acts a global regulator of growth and cell survival in response to environmental cues and is a member of the phosphoinositide 3-kinase (PI3K)-related kinase family. mTOR signalling plays a crucial role in the maintenance of cellular homeostasis as demonstrated by its deregulation in cancer, obesity, epilepsy and type 2 diabetes [16]. Target of Rapamycin (TOR) was first identified in budding yeast when genetic screening of the cytotoxic effects of the bacterial macrolide, Rapamycin, resulted in the discovery of two mediator proteins, termed; TOR1 & TOR2 [17]. Following shortly after this, the mammalian counterpart was purified by Brown, Sabatini & Sabers, separately, within a year of each other [18-20]. It is present in 2 discrete complexes; mTOR complex 1 (mTORC1) & mTOR complex 2 (mTORC2) in which mTORC1 is a large protein complex made up of 6 known proteins; mTOR, GβL (mammalian lethal with SEC13 protein 8 or mLST8), Raptor (Regulatory associated protein of mechanistic target of rapamycin), DEP domain-containing mTOR-interacting protein (DEPTOR), the Tti1/Tel2 complex and the mTORC1 inhibitor protein, PRAS40 (Proline-rich Akt substrate 40kDa) [16, 21]. This complex integrates multiple signals that sense the availability of growth factors, nutrients and energy to promote and direct cellular growth and catabolic processes to maintain cellular homeostasis [22-23]. The mechanism by which rapamycin interacts to inhibit this protein complex is not well known, however, what is known is that it forms a complex with intracellular 12-kDa KF506-binding protein (FKBP12) which interacts directly with the mTOR to inhibit its function when part of mTORC1 but not mTORC2. The action of inhibition and binding by this FKBP12- Rapamycin complex is not yet clear but studies suggest that it may compromise the structural integrity of mTORC1 [24] and cause allosteric reduction in the activity of its kinase domain [18]. DEPTOR was identified in 2009.

One of the less understood mechanisms of mTOR activation is amino acid sensing, which is profoundly important not only in cancer but in neurological diseases such as Focal Epilepsies [25]. What is understood about this pathway is that heterodimer the heteropentameric protein complex termed Ragulator, encoded by MAPKSP1, ROBLD3 and c11orf59 genes, occurs where Ragulator acts as both an anchor for RAGs to the lysosomal surface and as the GEF (Guanine nucleotide exchange factor) protein for RAGA/B [27]. Glutaminolysis was also shown to drive GTP loading of RAGB upstream of Ragulator/RAG and it was shown that inhibition prevented GTP loading and so inhibited mTORC1 activation [28]. This RAG/Ragulator complex causes the translocation of mTORC1 to the lysosomal surface where it is active. When mTORC1 was constitutively targeted to the lysosomal surface, it was sufficient to create a pathway independent of both the RAG/Ragulator complex and amino acid sensitivity, but still required the action of RHEB-GTP to function. This demonstrated how this interaction between mTORC1 and RAG/Ragulator is vital for amino acid signalling [21]. Following this, Gap activity towards Rags (GATOR) protein complexes were identified using human embryonic kidney cells (HEK293T) with FLAG-tagged RAGB with a chemical cross-linker in order to stabilise any coimmunoprecipitated complexes which form regardless of their weak interactions with one another. This was then analysed by mass spectrometry and GATOR complex proteins were identified and RNAi (RNA interference) of these newly identified proteins, lead to inhibition of amino acid induced activation of mTORC1 [29]

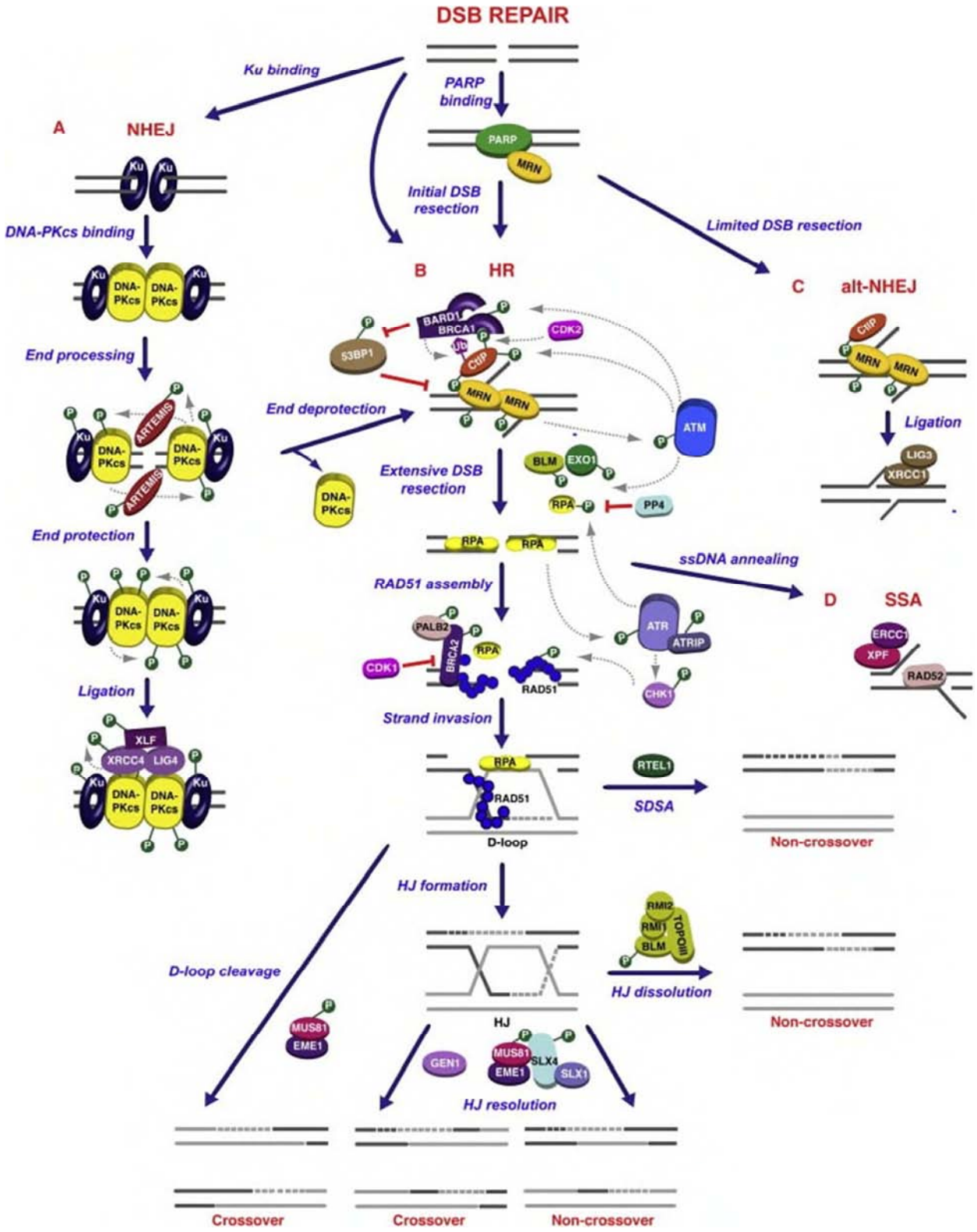

Figure 3. *During a DSB event several alternative pathways are positively selected for depending on the mitotic phase of the cell. During A) Ku promotes NHEJ by DNA-PKc recruiting and subsequent protection of DNA ends. This form of DSB repair is inherently error prone and occurs throughout the cell cycle with an elevation during G2/M phase [26]. Alternatively, B) HR occurs mostly during S phase after DNA replication where homologous sequences are available, This BRCA2 and RAD51 mediated strand invasion is nearly errorless and can be resolved either causing crossover events or non-cross over events. PARP1 which initiated HR also acts in direct competition with Ku70/Ku80 heterodimer. C) shows alternative-NHEJ which is carried out by CtIP and MRN during G1 phase. D) shows the SSA as an alternative pathway when homologous ssDNA sequences are directly annealed by RAD52 [8, 26]. The preference of repair depends upon the cell cycle and transient concentrations of proteins at the time, for example HR is almost non-existent in cells during G1 phase while no DNA replication has occurred (image adapted from [8]).*

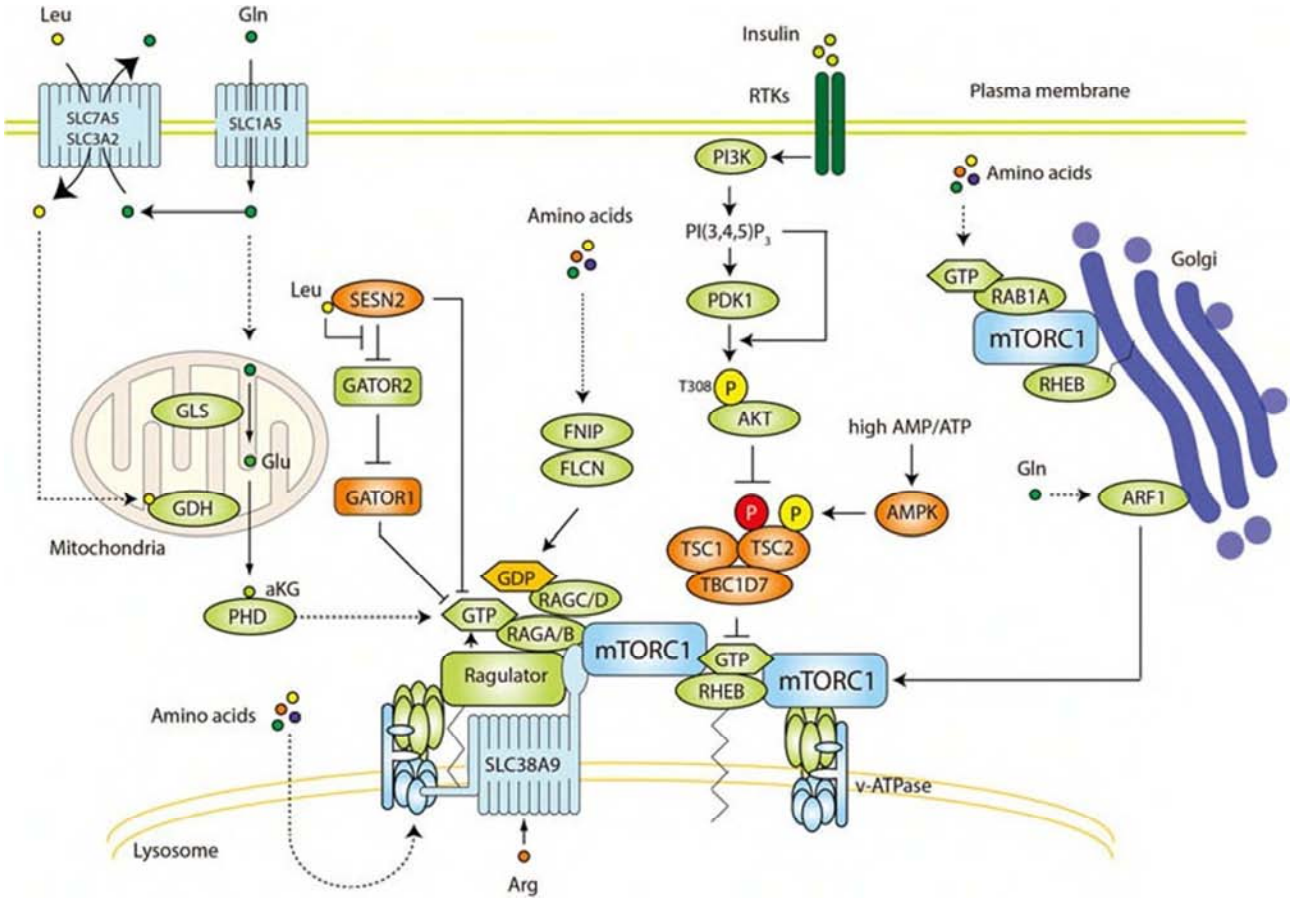

Figure 4. *The multiple upstream inputs that coordinate and regulate the mTOR pathways in human somatic cells is an intricate web which is ever expanding. TSC1/2 acts as the main upstream regulator of the mTORC1 pathway, processing signals from multiple stimuli to effect the activity of mTORC1 through its GAP (GTPase activating protein) activity towards RHEB, thus effecting the translocation of mTORC1 to the lysosomal surface, the site of mTORC1 activation. (Image adapted from [27]).*

4. mTORC1 Signalling Pathway

When assessing the upstream signals which affect the mTOR pathway, the developed knowledge of mTORC1 is better understood than that of its counterpart. Its complexity in upstream signals compliments its known nature in the cell as global regulator essential for maintaining homeostasis. It, as far as current understanding extends, integrates signals from five main extra- and intra-cellular cues including Hypoxia and oxygen sensitivity, cellular stress signals, energy status, growth factors and amino acid levels to adjust a multifaceted variety of cellular processes such as lipidogenesis, protein synthesis, as well as inhibition of autophagy and lysosomal biogenesis [16]. Through the numerous inhibitions and initiations of catabolic and anabolic processes respectively, mTORC1 is also a major promoter for cell-cycle progression [18] as shown below in figure 3. mTORC1 can be activated by growth factors such as insulin as a downstream target of PI3K signalling. Insulin binds to insulin receptors and recruits insulin receptor substrate (IRS), activating the PI3K-PDK1-Akt pathway to inhibit TSC1-2 complex through the phosphorylation of a number of residues, Thr1462, Ser939 & Ser98 1[36]. This results in RHEB-GTP binding to and activation of mTORC1 on the lysosomal surface. Converesely, a high AMP/ATP ratio stimulates the phosphorylation of AMPK (AMP activated protein kinase) to activate TSC1-2 complex [27].

One of the less understood mechanisms of mTOR activation is amino acid sensing, which is profoundly important not only in cancer but in neurological diseases such as Focal Epilepsies [21]. What is understood about this pathway is that amino acids promote the activation of RAG GTPases (RAG A/B/C/D) to recruit mTORC1 to the lysosomal surface whereby it interacts with RHEB. RAGA/B forms a heterodimer with RAGC/D and is subsequently activated through amino acid dependant modulation of its guanine nucleotide binding status. When RAGA/B is GTP bound and RAGC/D is GDP bound, the heterodimer is active [27]. A protein interaction between the RAG heterodimer the heteropentameric protein complex termed Ragulator, encoded by MAPKSP1, ROBLD3 and c11orf59 genes, occurs where Ragulator acts as both an anchor for RAGs to the lysosomal surface and as the GEF (Guanine nucleotide exchange factor) protein for RAGA/B [27]. Glutaminolysis was also shown to drive GTP loading of RAGB upstream of Ragulator/RAG and it was shown that inhibition prevented GTP loading and so inhibited mTORC1 activation [28]. This RAG/Ragulator complex causes the translocation of

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GATOR forms two distinct subcomplexes GATOR1 & 2, GATOR1 being comprised of DEPDC5, Nprl2, and Nprl3 where as GATOR2 contains the subunits; Mios, WDR24, WDR59, Seh1L, and Sec13. Studies show that GATOR1 subunit inhibition created resistance to amino acid deprivation in mTORC1 activation whereas epistasis analysis revealed that GATOR2 negatively regulates a subunit of GATOR1; DEPDC5 [29]. Mutations in this subunit have been implicated in both Hepatitis C carrier hepatocellular carcinoma patients [30] and Astrocytic Tumours via microsatellite array analysis of its gene loci on chromosome 22 [31]. This mTORC1 repressor has also become the focus of research involved in familial focal epilepsy (FFE) and focal cortical dysplasia (FCD), where it was shown that out of 50 sequenced genomes of subjects with FFE with or without FCD, 11% contained GATOR1 subunit mutations, most prominently in DEPDC5. This was enriched further when studying FFE with FCD in which 28% of all sequenced mutations were found in the GATOR1 subunits [25]. Regulators of this GATOR2 subunit are all driven by amino acid availability, such as the Sestrin protein, SESN2, which acts as a leucine sensor in the cytoplasm where SESN2-Leucine is a negative regulator of both GATOR2 and of RAGA/B as a GDP dissociation inhibitor [32].

The CASTOR proteins were identified in 2016 as Arginine sensors for the mTORC1 after a lysosomal transmembrane protein, SLC38A9 was identified as an arginine sensor required to activate mTORC1 [33]. CASTOR1 is another arginine sensing protein complex which forms a homodimer with itself or a heterodimer with CASTOR2, forming a complex with and sequestering GATOR2 activity. Arginine is required to disrupt the GATOR2-CASTOR1 interactions and subsequently activate and localise mTORC1 to the lysosomal surface [32]. This is an example of how amino acid concentrations can dictate mTORC1 activity.

DEPDC5 Crispr-Cas9 modification in HEK293T cells expressing Flag-tagged versions of the protein allowed anti-flag-tagged immunoprecipitation of co-precipitated proteins to GATOR1. Results showed that GATOR2 and a novel protein complex named KICSTOR; consisting of proteins encoded by KPTN, ITGF2, SZT2 and C12orf66 formed a complex with GATOR1 at endogenous levels [34]. Transient expression analysis of recombinant versions of each subunit was carried out and found that products of KPTN & ITFG2 form a heterodimer and interact with C12orf66 in a SZT2 dependant, non- competitive manner. *In vivo* models showed increased mTORC1 activity when lacking SZT2 and was shown to be necessary for GATOR1 interactions with its associated substrates on the lysosomal surface of cells. Indicating that KICSTOR acts a downregulator of mTORC1 and is another site of mutation in human disease [34]. Further study revealed SZT2 recruits small quantities of GATOR1 and GATOR2 to form a SZT2 orchestrated GATOR complex (SOG) that plays a central role in determining GATOR-dependant nutrient sensing and GATOR2 suppression of mTORC1 through SESN recruitment where SZT2 deficiency resulted in amino acid insensitive constitutive mTORC1 signalling [35]. Previous analysis of SZT2 gene showed expression predominantly in the brain and knockout studies in mice revealed low seizure thresholds, potentially enhancing epileptogenesis [36]. Immunoflourescent analysis on *in vitro* primary neuronal cells demonstrated that the product of KPTN (Kaptin) associates with actin cytoskeletal structures and that subsequent mutational inputs causes loss of function. Linkage analysis and whole exome sequencing revealed mutations in KPTN caused a syndrome characterised by neurodevelopmental delay, seizures and macrocephaly, suggesting that it plays a necessary role in human neuromorphogenesis as well [37].

As previously discussed, downstream mTOR signalling contributes to nutrient sensing and are necessary for cell growth and subsequent progression through the cell cycle for cell division. The most well-characterised process controlled by mTORC1 is protein synthesis where by mTORC1 phosphorylates translational regulator eukaryotic translation initiation factor 4E (elF4E) binding protein 1 (4E-BP1) which leads to promotion of protein synthesis through dissociation of 4E-BP1 from elF4E and the subsequent recruitment of elF4G to the 5' cap, allowing the initiation of translation. Alongside this, mTORC1 phosphorylates S6 kinase (S6K1) which recruits elF4B to the pre-translational initiation complex and enhances elF4A RNA helicase activity [38].

mTORC1 pathway and its role in DNA damage repair.

In recent years, convincing data has emerged that suggests mechanistic links between signalling through the PI3K-Akt-mTOR cascade and components of the DDR. These interactions occur at multiple signalling tiers, and impact on DNA repair in a variety of ways. For example, mTORC1/mTORC2 dual inhibition has been shown to sensitise T-cell acute lymphocytic leukaemia and rhabdomyosarcoma cells to DNA damaging agent by downregulation of FANCD2, a gene involved in the Fanconi anaemia pathway, a key DNA repair mechanism [9]. Akt has also been shown to mediate the repair of ionising radiation induced DSBs in DNA. It was demonstrated that though some cancer cell lines became radiosensitive to rapamycin

and ionising radiation, in non-responsive cell lines to rapamycin and ionising radiation induced radiosensitivity increased activation of the PI3-K/Akt survival pathway and led to enhanced repair of DSBs in the DNA and increase radio-resistance. Combined *in vitro* targeted inhibition of both Akt and mTORC1 lead to restored radiosensitivity in these cell lines [39].

Certain PI3K/mTOR inhibitors can also inhibit ATM and DNA-PK mediated DNA damage responses in non-small cell lung cancer (NSCLC) cell lines; where both HR and NHEJ were blocked by a dual inhibitor (NVP-BEZ235) in an Akt independent manner, in addition to the phosphorylation of ATM targets and inhibition of G2/M cell cycle progression [40]

Recent Studies have also revealed that mTOR may also play a crucial role in post- transcriptional regulation of two DNA repair proteins in long lived mice phenotypes – O-6-methylguanine-DNA methyltransferase (MGMT) and N-myc downstream-regulated gene 1 (NDRG1) through the post-transcriptional gene expression complex - CCR4-NOT, which lies downstream of mTORC1 pathway. Down-regulation of mTOR enhanced expression of MGMT and NDRG1 and instigated mTOR as a post-transcriptional regulator of DNA repair through specific alteration of CCR4-NOT [41].

KICSTOR subunits have also been instigated to play a potential role in the DDR mechanisms through their domain homology with key DDR proteins such as c12orf66, which shares a gelsolin-like domain (a domain which plays a role in actin nucleation which activates in response to multiple cellular stresses such as heat shock or protein misfolding). Recent studies have revealed that DNA damage is another cellular perturbation which initiates nuclear actin filament generation and that this response requires 2 nucleation factors - Formin-1 and Spire-1/2, which accumulate in the nucleus after DNA damage as part of the DDR. Depletion of Formin-1 or the actin nuclear import factor, importin-9, revealed an increase in DNA DSBs linking actin filament formation in the nucleus and the DDR [42].

Swiss modelling of the SZT2 sequence reveals an alpha-beta domain with homology to the von Willebrand Disease-like domain of Ku70 mapped on the N-terminus. Mutagenesis of this domain led to i6ncreased cell survival following IR treatment through the regulation of apoptotic factors such as ATF2 (activating transcription factor 2) regulating genes to the site of DNA damage [43].

5. Summary

The recent interest in KICSTOR and its subunits rises, the likelihood that it plays a role beyond amino acid sensing and potentially in the regulation of DNA damage repair sensing is entirely plausible. As inhibitors of PI3K/Akt/mTOR have been shown to radio- sensitise cancer cells and inhibit DNA repair and given the protein homology of SZT2 and c12orf66 with proteins intrinsic to DDR mechanisms, the further study of this pathway in respects to DNA damage could prove invaluable to future cancer therapies. With the widespread use of DNA-damaging agents as chemotherapies, knowledge of the interplay between these processes could create novel opportunities or inform existing strategies while expanding the overall knowledge of a ubiquitously expressed, multifaceted signalling pathway intrinsic to cell survival, growth and proliferation.

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