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CHROMATIN STRUCTURE AND DNA DAMAGE RESPONSE

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Genomic integrity is constantly exposed to the products of metabolic activities and environmental processes that can induce DNA damage. A well-organized network of signaling cascade, designated as DNA damage response (DDR), encompasses systems of damage detection, cell-cycle check-point activation and repair mechanisms. The DNA damage pathways involve not only naked DNA strands but also higher-order chromatin components, such as histone variants and heterochromatin proteins. Any impediment of this regulation process may cause extensive damage and trigger the growth of tumours. The alterations in chromatin architecture occur during transcription and replication and are required to provide the accessibility of proteins to DNA strands. There is increasing evidence that DNA repair is also accompanied by the chromatin remodeling, particularly in the case of efficient detection and repair of DSBs where chromatin structure and nucleosome organization represent a significant barrier.

Keywords: *DNA damage, ATM, ATR, chromatin, DSBs, DDR.*

СТРУКТУРА ХРОМАТИНА И ОТВЕТ НА ПОВРЕЖДЕНИЕ ДНК

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Геномная целостность постоянно подвергается воздействию продуктов метаболизма и процессов окружающей среды, которые могут вызывать повреждение ДНК. Хорошо организованная сеть сигнального каскада, называемая ответом на повреждение ДНК, охватывает системы обнаружения повреждений, механизмы активации и ремонта контрольных точек клеточного цикла. Пути репарации ДНК связаны не только с нитями ДНК, но также с компонентами более высокого порядка хроматина, такими как варианты гистонов и белки гетерохроматина. Любое препятствие этому процессу регулирования может привести к серьёзному повреждению ДНК и спровоцировать рост опухолей. Изменения в архитектуре хроматина происходят во время транскрипции и репликации и необходимы для обеспечения доступности белков к нитям ДНК. Появляется всё больше доказательств того, что репарация ДНК также сопровождается ремоделированием хроматина, особенно в случае эффективного обнаружения и восстановления двунитевых разрывов ДНК, где структура хроматина и организация нуклеосом представляют значительный барьер.

Ключевые слова: *повреждение ДНК, хроматин, ATM, ATR, двунитевые разрывы ДНК, ответная реакция на повреждение ДНК.*

Introduction

The cellular environment is continuously being challenged by the impact of endogenous factors, such as products of normal metabolism, as well as during DNA replication, transcription, and recombination, and exogenous or environmental factors, such as exposure to UV radiation, ionizing radiation, and treatment by various chemical agents [1]. All these factors often lead to the DNA lesions that might contribute to the various outcomes. Firstly, DNA damage might initiate malignant process; secondly, it is used to cure cancer and, thirdly, it can contribute to side effects of cancer treatment [2]. Depending on the challenge, different types of DNA damage such as DNA base damage, single stranded DNA (ssDNA), interstand crosslinking of DNA and DNA double-strand breaks (DSBs) can occur. Thus, the genomic DNA of mammalian cells evolved a robust genome surveillance system to maintain DNA integrity through activation of alternative cell fate pathways [3].

DNA damage signaling pathway

Induced DNA damage initiates subsequent activation of the signaling pathway known as the DNA damage response (DDR) that represents a multistep process involving a network of DNA damage response protein substrates [4]. Once DSB occurs, the sensor substrates recognize and detect the DNA damage site with further transmission of the DNA damage signal to the transducers, proteins that promote activation of various protective pathways starting with initiation of cell-cycle checkpoints, mechanisms that arrest the cell cycle division, followed by cell cycle arrest, DNA repair or apoptotic programs via downstream effectors [5]. DDR is generated by trimetric highly conserved MRN (MRE11-RAD50-NBS1) protein complex that is involved in both modes of DSB repair, non-homologous end joining (NHEJ) and homologous repair (HR) that requires the undamaged homologues DNA template to restore the original DNA sequence, and consequently restricted at S phase and G2 when the sister chromatid is available. The initial stage of implication of MRN complex in DSB happens due to nuclease activity and DNA binding capability that belong to the MRE11 and is partially determined by MRE11 and Rad interaction [5]. Nbs1 protein is responsible for nuclear localization and placement of the complex at the DSB site via its direct interaction with protein histone variant H2AX. Furthermore, the enrichment of MRN complex at the DSB ends promotes the recruitment and activation of central DDR signaling protein kinase Ataxia Telangiectasia Mutated (ATM) [4].

PIKK family members

ATM and related kinases ATR (ATM and Rad3-related) and DNA-PK (DNAdependent protein kinase) refer to phosphorylatidylinositol 3-kinase–like (PIKK) family of serine/threonine protein kinases, and all are implicated in DNA DSB response pathways [4]. The members of PIKK family possess noticeable similarities in the architectural structure and share sequence homology, particularly in the following domains such as C-terminal domain, flanking FAT (FRAP–ATM–TRRAP) and FATC (FAT C-terminal) domains [6] (Figure 1-1) known to be involved in the regulation of kinase activity. The N-terminal

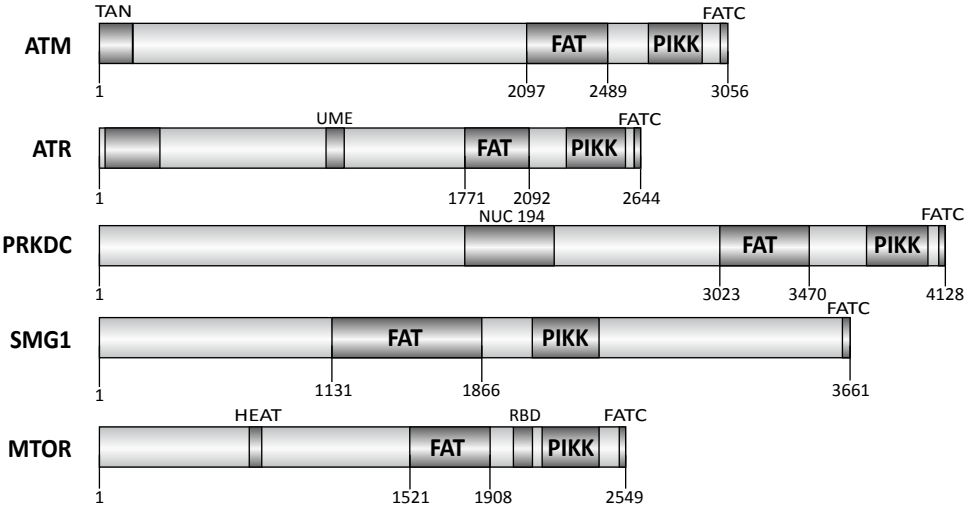

Figure 1-1: Schematic presentation of domain architecture of PIKK family members. Displayed proteins are: ATM (ataxia-telangiectasiamutated), ATR (ataxia–telangiectasia and Rad3 related), PRKDC (DNA-dependent protein kinase catalytic subunit; DNA-PKcs), SMG1 (suppressor of morphogenesis in genitalia), MTOR (mammalian target of rapamycin).Conserved domains are: FAT (FRAP–ATM–TRRAP domain), FATC (FAT C-terminal domain), PIKK (phosphatidylinositol 3-kinase-related kinase domain), TAN (Tel1/ ATM N-terminal motif), ATRIP (ATR interacting protein),UME (UVSB PI3 kinase,MEI-41 and ESR1 domain), NUC194 (domain B in the catalytic subunit of DNA-dependent protein kinase), RBD (rapamycin binding domain), HEAT (Huntingtin, elongation factor 3, protein phosphatase 2A, and the yeast kinase TOR1-like repeat) [8].

presents poorly conserved region among PIKK family members and contributes to the protein-protein interaction with various substrates [7].

Ataxia Telangiectasia Mutated (ATM) is one of the central kinases that activated by formation of DNA DSBs. ATM molecule transforms from inactive dimer into an active monomer by autophosphorylation on Ser1981 as a result of DNA DSBs [9]. The recruitment of activated ATM at sites of DNA DSBs occurs through its binding to C-terminus of NBS1 of MRN complex. This interaction promotes further kinase activity of ATM [10].

Proteins involved in DNA damage repair pathway

Following its recruitment at sites of DNA DSBs, activated ATM protein kinase rapidly phosphorylates the histone variant H2AX on Serine 139 (noted as γH2AX). Alteration of histone dynamics in DNA repair is one of the crucial moments in DDR. DSBs induce not only histone modifications but also lead to the histone variants involvement in the DNA repair process. Thus, generation of γH2AX foci amplifies the DNA damage signal and promotes further recruitment of DDR proteins at the breakage sites. Accumulation of γH2AX foci in the regions flanking the lesion serves as an indicator of DSB presence and a marker for DDR activation [11].

This suggests that ATM kinase initiates a cascade of DDR factors to provide effective DNA damage repair [12]. Moreover, activated form of ATM exposes protective properties through regulation of cell cycle. Thus, in response to the induction of DNA DSBs ATM triggers activation of the G1/S cell cycle checkpoint and thus restrains cells with damaged DNA from entering the S-phase. This defensive mechanism is initially mediated through direct phosphorylation of p53 on serine 15 by ATM kinase. Tumour suppressor protein p53 was reported as one of the first downstream substrates targeted by ATM kinase. Additionally, stabilisation of p53 can be achieved via phosphorylation of p53 on serine 20 by checkpoint kinase (CHK2), another key target substrate of ATM. This phosphorylation prevents p53 from Mdm2- mediated ubiquitination and degradation [13]. Furthermore, ATM rescues p53 from degradation by direct phosphorylation of Mdm2. The activated form of p53 accumulates in the nucleus and acts as a transcription factor. As a result of this function, p53 stimulates the expression of genes implicated in cell cycle activity as well as several genes contributing to cell apoptosis [14]. Further contribution of ATM kinase to prevent the synthesis of damaged DNA is fulfilled through its S-phase checkpoint functions. Thus, phosphorylated by ATM CHK2 kinase lead to ubiquitination and degradation of the S-phasepromoting phosphatase Cdc25A which in adequate cellular microenvironment promotes S-phase progression via activation of the cyclin-dependent kinase 2 (Cdk2) that is required for DNA synthesis [15].

Role of ATR kinase

Alongside ATM, the ATR signaling pathway also takes part in enforcement of the intra-S-phase checkpoint in the presence of impaired replication fork. The pathway is governed primarily by ATR, and may involve members of the Rad family of checkpoint proteins as damage sensors and as scaffolds for the assembly of checkpoint signaling complexes. The regulation of this pathway prevents mitotic catastrophe that results from incomplete or inaccurate DNA replication, and orchestrates high-fidelity DNA repair through homologous recombination [16].

Similar to ATM, ATR refers to central kinases implicated in DDR. However, unlike ATM, activation of ATR kinase is provoked by single strand DNA (ssDNA) damage that occurs, for instance, at stalled replication forks. Thus, interaction of ATRIP (ATR-interacting protein) 51 and the N-terminus of ATR induce activation and localization of ATR kinase to damage sites. Once activated, ATR exerts its DNA repair properties via recruitment of target substrates [17-19]. Many of ATR functions are achieved through CHK1 kinase, the specific ATR downstream substrate. Through CHK1- mediated degradation of Cdc25A phosphatase ATR inhibits progression of DNA replication thus ensuring sufficient time to resolve the stress condition [20-22]. Moreover, ATR also regulates G2/M cell cycle checkpoint by preventing mitotic entry in response to DNA damage [23].

ATM and ATR

Despite the differences in the activation of ATM and ATR signaling pathways, they share the plethora of downstream target substrates. Both kinases possess similar substrate specificity and phosphorylate their target substrates on serine or threonine residues that precede glutamine residues, termed as SQ/TQ-motifs [24]. It was reported, that more than 700 ATM/ATR putative target substrates have been identified as a result of a large scale proteomic study. Therefore, the cellular functions of these protein kinases partially

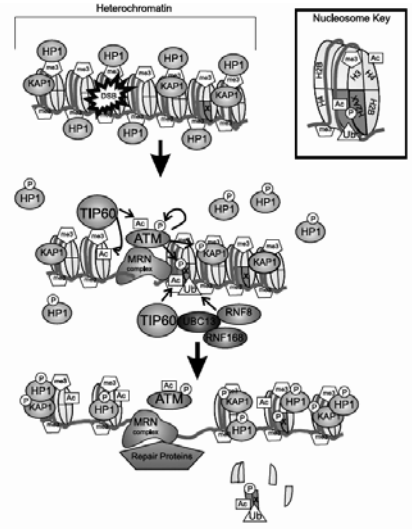

Figure 1-2: Alterations in heterochromatin architecture as a result of DNA DSBs. Triplicate MRN complex recognizes and accumulates at the DNA DSBs sites with further recruitment of ATM protein kinase. As a result of HP1α release from H3K9me3 the vacant place is occupied by Tip60 and ATM complex that promotes the involvement repair proteins. Acetylation of ATM by Tip60 leads to the ubiquitination of ATM by RNF8 and RNF168 ubiquitin kinases and thus contributes to the amplification of DNA damage signal [3].

overlap, and the failure of one pathway might be compensated by other pathway [25]. ATM and ATR confer a range of biological functions including cell survival, proliferation, differentiation, metabolism and motility. Moreover, a significant body of research suggests PIKK family members are linked to tumorigenesis and refers these kinases as attractive putative targets for cancer therapy. A functional interplay between ATM and ATR contribute to the maintenance of genomic stability after DNA damage by regulating cell cycle progression and DNA repair [17, 26]. Thus, ATM as a known tumour suppressor gene is found mutated in a wide range of human cancers such as breast, lung, colorectal and hematopoietic cancers [27]. Initially, ATM was identified as a gene defective in the autosomal recessive human hereditary disorder ataxia–telangiectasia (A–T). This multisystem condition, caused by a defect in ATM function, is characterized by oculocutaneous telangiectasia, progressive cerebellar ataxia, immunodeficiency, radio-sensitivity and predisposition to malignancies [28]. ATR-deficiency impedes the viability of multicellular organisms [29]. Furthermore, it was reported that ATR depletion in adult mice manifested in the defect of tissue homeostasis as a result of restricted proliferation, whereas ATR activity is essential to maintain the proper DNA replication for all proliferating cells and thus to ensure genomic stability. ATM and ATR-mediated DNA damage pathways encompass detection of DNA lesions, repair mechanisms, cell-cycle check point induction and apoptosis. However, for fulfillment the above mentioned functions it is important to consider the complexity of chromatin structure. It is noteworthy, that sensitivity of DNA to damage and the kinetics of repair can be determined by chromatin density [30].

Impact of chromatin structure on DNA damage response

As genomic material is presented by nucleo-protein complex, consequently DNA template and proteins involved in its compaction process are implicated in DNA damage response pathways [3]. The interest in the correlation between DNA DSB induction and alterations in chromatin structure was observed throughout the decades. DNA double-strand break (DNA DSB) refers to one of the most deleterious types of DNA lesions with destruction of both strands of DNA molecule [31]. The failure of DSB lesion repair can lead to translocations, inversions, deletions and chromosomal abnormal rearrangements. All these alterations might lead to accumulation of the vitally dangerous mutations or promote the process of tumorigenesis.

Topological organization of chromatin has impact on all nuclear processes, including DNA damage response. Thus, the difference in chromatin compaction in euchromatin and heterochromatin reflects on the both damage sensitivity and repair. Heterochromatin decondensation is crucial for inducement of DNA damage signal amplification as well as for provision of access for DNA repair proteins to the breakage sites [32]. Thus, manipulation of chromatin structure dictated by ATP-dependent chromatin remodeling complexes and histone-modifying enzymes is involved in any DNA repair process to allow access to DNA. Heterochromatin condensation impedes the DNA repair, and as a result heterochromatic double strand breaks (DSBs) recover with slower kinetics and less efficiency compare with euchromatic DSBs [4].

Chromatin complexity and DNA damage response

It was demonstrated that hetero-chromatization is the principal criteria of ATM involvement, and implication of ATM in DNA damage response is determined by chromatin complexity rather than by damage complexity. Thus ≤25% of DSBs require ATM-signaling for repair where ATM kinase facilitates DSB repair by affecting the heterochromatin–building components such as KAP-1, HP1α, and/or Tip60 (Figure 1-2) (Kurz and LeesMiller, 2004). Recent studies demonstrate that HP1α has a dual role in DDR. Thus, phosphorylated HP1α by casein kinase 2 (CK2) temporarily removes HP1α from H3K9me3 leading to the heterochromatin de-condensation and providing access to DNA repair proteins [3]. As a result of HP1α release from heterochromatin, the space vacated by H3K9me3 is occupied by complex of Tip60 and ATM that gains activation and initiates DDR by further phosphorylation of downstream substrates [3].

Alternatively, it was reported that chromo-shadow domain determines the recruitment of HP1α to the DSBs sites and provides accumulation of HP1α at the sites of heterochromatic perturbation by amplifying DNA damage signal whereas chromodomain and H3K9me3 participates in the disassociation of HP1α and promotes the chromatin decondensation [3]. Moreover, enrichment of HP1α at pericentric heterochromatin provides stabilization and integrity of sister chromatids as well serves as recognition site for mediators of the DNA damage response pathway. Thus, bimodal behavior of HP1α at the DNA DSBs sites is recognized as a crucial mediator of chromatin relaxation and at the same time participates in the maintenance of genomic stability and is important factor for the completion of DNA repair [3]. Experimental evidence demonstrated that chromatin relaxation is partially determined by ATM-mediated phosphorylation of transcriptional repressor 56 KAP1 (KRAB-associated protein; TIF1b; TRIM28). KAP1 is an abundant nuclear protein that binds in a sequence-dependent manner to KRAB domains to contribute to the heterochromatin construction through association with heterochromatin-building factors such as HP1, histone deacetylases (HDACs), SET-domain histone methyltransferases, and ATP-dependent chromatin remodelers [4]. ATM-KAP1 association for

chromatin decondensation is required during G2 phase as well as G0/G1 cells for efficient DSB repair. However, heterochromatic DSB repair during DNA replication was demonstrated to be ATR-dependent. Therefore, the phosphorylation of KAP1 during this phase of cell cycle is mediated by ATR kinase. More recently, it was reported that mutation or deletion of heterochromatin-related factors (KAP-1, HP1, HDAC1/2, and Suv39H1/2) impedes the function of ATM kinase in DNA repair [4]. This data demonstrates the specificity of ATM to induce chromatin relaxation within heterochromatic DSBs [4]. Alongside the contribution to the enhanced DNA damage signalling and more efficient repair, the role of heterochromatin proteins is also directed on reestablishment of chromatin structure following DNA damage. According to the recent reports, the deletion of KAP1 led to the increased DNA sensitivity thus highlighting the function of the protein in the maintenance of the genomic stability [4]. Hence, through regulation of the transcriptional machinery access to the DNA strands and modulation of dynamic chromatin architecture, chromatin-associated proteins orchestrate a range of critical cellular events including DNA repair and a promotion of malignant transformation and progression [3].

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