ATM: awakening the guardian angel or drugging the “absolute tailor master”

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Abstract

ATM is versatile member of DNA damage repair mechanism, involved in monitoring the genomic integrity. Any damage done to DNA relentlessly challenges this guardian’s credibility. This multitalented mediator comes into action to recapitulate faithfully the derailed genomics. Double stranded breaks are a consistent threat and actively implicated in tipping the scales of cancer in favor of genomic instability. Therefore it holds great potential in safeguarding and resolving efficiently abrupt aberrations.

According to the increasing sophisticated information, ATM silencing and stimulation, both are being evaluated for a better therapeutic intervention. In this review we will analyze both paradigms of rational drug design. Taking into consideration, the emerging snapshot of push and pull between diametrically opposed roles of ATM, there is an urgent requirement to tailor therapies with promising clinical outcomes.

Introduction

Ataxia telangiectasia (A-T) is a neurological disorder associated with degeneration in the cerebellar part of the brain that controls motor movements and speech. About 20% patients with A-T develop cancer, especially acute lymphocytic leukemia (ALL) or lymphoma. Because of the wide clinical heterogeneity A-T is often difficult to diagnose in children. Recently, a research group reported an unusual case of three year old boy affected by A-T who presented exclusively with extensive cutaneous granulomalosis. This feature is another addition to the list of symptoms of A-T. Ataxia telangiectasia portrayed multifaceted roles according to the current understandings and holds a key position and an authoritatively acclaimed key player involved in pathogenesis [1-3].

Using a positional cloning strategy, Savitsky et al identified a gene that they designated ATM. A partial ATM cDNA clone of 5.9 kb identified a 12-kb band on Northern blot [4]. The predicted 3,056-amino acid protein had molecular mass of 350.6 kD and showed significant sequence similarities to several yeast, Drosophila, and mammalian phosphatidylinositol 3-kinases (PI3Ks) [3]. In this particular review we will focus on the convergent and divergent trends of ATM that will advance personalized medicine principles into healthcare management tools for individuals and populations.

ATM: changing landscape of fast flying kinase

The recognition and signalling of DNA damage engages miscellany of proteins including the protein kinase ATM mutated in A-T. It is well acclaimed that appropriate DNA damage response of cells necessitate amplification of the ATM-dependent damage signal by recruitment of DNA damage checkpoint mediators to the site of chromatin.

ATM kinase is activated in the closer proximity of the break and is tethered to the break site by the Mre11/Rad50/Nbs1 (MRN) complex where it is fully activated. Earlier it was interpreted that activation process involves autophosphorylation on three sites (S367, S1893 and S1981) and acetylation of the break and is tethered to the break site by the Mre11/Rad50/Nbs1 (MRN) complex where it is fully activated. Nonetheless, recently Kozlov and co-workers identified a new ATM phosphorylation site, pT1885, and an additional autophosphorylation site, pS2996, that is remarkably responsive to DNA damage [5]. DNA-damage response (DDR) machinery exerts tumor suppressor activity in gliomas [6].

Earlier it was presumed that ATM is nuclear and executed DNA damage response in nucleus but some of the recent documentations are contradictory. According to the current snapshot, after induction of DSBs, ATM translocates from the nucleus and stimulates tumor necrosis factor receptor-associated factor 6 (TRAF6) that
consequently triggers auto-polyubiquitylation of TRAF6 and the polyubiquitylation of the IKK adaptor ELKS, respectively. It is also interesting to note that ubiquitylation promotes the assemblage of signalosomes containing the kinase TAK1 (transforming growth factor b-activated kinase 1) (Fig. 1). These signalosomes mediate activation of the cytosolic IKK complex, which modulates NF-κB-dependent reprogramming of a neoplastic cell [7]. In the next section we will point out some principle differences in context and tissue dependent behavior of ATM.

**ATM activation: watch dog**

ATM activation plays a role as a barrier to tumorigenesis. Cells deficient in ATM are more susceptible to DNA damage [8]. Despite the fact that majority mutations in the ATM gene result in truncated proteins, drugs that allow read-through termination codons represent other avenues to restore/regain expression of full length functional ATM protein. Indeed it has been shown recently that treatment with geneticin and/or gentamicin produced detectable read through expression of a functional ATM protein in fibroblasts [9].

ATM once inactivated is unable to mediate the efficient repair of the genome. It has already been established that ATM activities are severely impaired in a wide range of molecular disorders. Various anomalies at transcriptional and translational level influence the expression of full length protein product of ATM. Any aberrant protein product of ATM is incompetent to undertake the process of autophosphorylation and contribute in the DNA repair. There are previous reports of retrieving the hampered activities of ATM by antisense morpholino oligonucleotides (AMO) which block the splice site mutations and there is no illegitimate skipping thus maintaining the integrity of ATM mRNA transcript. Similarly nonaminoglycosides that causes read through the stop codons which restores the normal protein length of ATM [9-11].

It is well documented that synergistic administration of drugs results in ATM pathway activation and chromatin remodeling [12]. It has recently been revealed that nitrogen permease regulator 2 (NPRL2), a tumor suppressor gene is involved in activation of ATM and potentiates the efficacy of cisplatin. It was observed that disruption of NPRL2 resulted in the loss of cisplatin activity [13].

On a parallel statement, selenium supplementation suppresses susceptibility of colorectal and other cancers. Selenium treatment enhances the interaction between hMLH1 and hPMS2 proteins, a heterodimer critical for DNA repair, in an ATM dependent fashion [14]. It is intriguing to note that functional inactivation of ATM is consequential to DNA replication-dependent hyperactivation of DNA-PK in camptothecin (CPT)-treated cells and striking CPT hypersensitivity. Furthermore, concomitant inhibition of ATM and DNA-PK partially retrieved CPT resistance, suggestive of the fact that activation of DNA-PK is proapoptotic in the absence of ATM [15].

Activation of the DNA damage response by polyphenols might abolish the oncogenesis. Additionally, testosterone, when combined with curcumin, may have antineoplastic influence on the progression of prostate cancer [16]. Similarly, genistein is also involved in a robust activation of ATM. Contrarily bioflavonoids including daidzein and biochanin A, did not induce either phosphorylation of p53 or ATM [17]. Outstanding activation was observed in p53 after treatment with camptothecin and doxorubicin, a downstream target of the activated ATM pathway [18, 19]. Recently our lab has focused on the involvement of ATM in enhancing the genomic stability by inhibition of a broad range of negative regulators in prostate cancer [20-23].
Inactivation: boarding the mysterious express

There are various cellular environments in which cells show refractoriness against a broad spectrum of therapeutic strategies. Recent concepts point towards the suppression of ATM in multiple molecular anomalies to counteract drug resistance. Consistent with this concept, activation of PKCα downregulates ATM, thus relieving ceramide synthase (CS) repression by ATM and enhancing apoptosis via ceramide generation \[24, 25\]. It is attractive to note that Aven (regulator of apoptosis) is actively involved in activation of ATM, thus triggering the DNA-damage response \[26\]. However, re-establishment of Aven blocks cisplatin-induced apoptosis by augmenting Bcl-xL protein levels in breast cancer cells \[27\].

Prostate cancer cell line was infected with antisense ATM expressing vectors against various domains of the ATM gene. Immunoblot analyses of cellular extracts from antisense ATM-transfected PC-3 cells displayed abrogated expression of the ATM protein \[28, 29\]. Impairment of ATM in p53-defective PC3 prostate cancer cells escalated cell cycle transition and sensitized cells to the killing effects of doxorubicin. Combinatorial approach seems to be appropriate as ATM knockdown with inhibitor UCN-01 further enhanced doxorubicin sensitivity in these cells. Paradoxically, the same strategy did not sensitize LNCaP prostate cancer cells, which has normal p53. LNCaP cells became more sensitive to doxorubicin or doxorubicin plus UCN-01 when there is a simultaneous suppression of p53 and ATM \[30\].

MicroRNA and ATM: gaze through the molecular lens

MicroRNAs (miRNA) are noncoding RNAs that regulate various cellular activities including proliferation and apoptosis. It has lately been acknowledged that miR-101 targets DNA-PKcs and ATM via binding to the 3′-UTR of mRNA of both of these kinases. Promoting robust expression of miR-101 capably declined the protein levels of DNA-PKcs and ATM in tumor cells and re-sensitized the tumor cells to radiation \[31\]. Another exciting piece of evidence was that ATM is the target gene of miR-181, which itself is mediated by TGF-β at the post-transcriptional level. Enforced expression of miR-181 resulted in ATM suppression \[32\]. ATM is actively involved in triggering miRNA biogenesis by phosphorylating KSRP, leading to enhanced interaction between KSRP and pri-miRNAs. This results in an increased KSRP activity in miRNA processing. Furthermore mutations of the ATM specific phosphorylation sites that harbored in KSRP, impaired its activity in regulating miRNAs \[33, 34\]. p63α is also a regulator of miRNA biogenesis that is phosphorylated by ATM \[35\].

There are some proteins which are overexpressed in neoplastic cells and dampen the DNA damage response. Wild-type p53-induced phosphatase 1 (Wip1) negatively regulates the ATM/ATR-p53 DNA damage signaling pathway. It is exciting to note that miR-16 specifically targets the mRNA of Wip1 and as a consequence negatively regulates the expression level of Wip1 \[36\]. Another exciting aspect recently documented is that pre-miR-630 blocked early DNA damage response, including the phosphorylation of the ATM \[37\]. ATM triggers TNFSF4 expression through miR-125b (Fig.2). It is clear from increasing complicated information that that miRNA-mediated gene regulation adds a new dimension to the DNA-damage response.

Conclusion

Detailed mechanistic insights of ATM are necessary to unravel the road blocks in the clinical management of cancer. While trying to get a step closer to individualized therapy it is unavoidable to have a complete molecular picture which needs to be completed in the future through experimentations and interpretations.
Abbreviations
3′-UTR; Three prime untranslated region
A-T; Ataxia telangiectasia
ALL; Acute lymphocytic leukemia
AMO; Antisense morpholino oligonucleotides
ATM; Ataxia telangiectasia mutated
ATR; Ataxia telangiectasia and Rad3 related
CPT; camptothecin
DDR; DNA-damage response
DNA-PKcs; DNA-dependent protein kinase, catalytic subunit
DSB; Double-stranded break
ELKS; Protein rich in amino acids E, L, K and S
hMLH1; human MutL homolog 1
hPMS2; human Mismatch repair endonuclease PMS2
(postmeiotic segregation increased 2)

References

Abbreviations
IKK; Inhibitor of kappaB kinase
KSRP; KH-type splicing regulatory protein
LNCaP; Lymph node carcinoma of the prostate
NF-kB; Nuclear factor kappa beta
NPRL2; nitrogen permease regulator 2
PI3Ks; Phosphatidylinositol 3-kinases
PKalpha; Protein kinase C alpha
TRAF6; Tumor necrosis factor receptor-associated factor 6
TAK1; Transforming growth factor b-activated kinase 1
TNFSF4; Tumor necrosis factor superfamily, member 4

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