MINI-REVIEW

Recently Emerging Signaling Landscape of Ataxia-Telangiectasia Mutated (ATM) Kinase

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Abstract

Research over the years has progressively and sequentially provided near complete resolution of regulators of the DNA repair pathways which are so important for cancer prevention. Ataxia-telangiectasia mutated kinase (ATM), a high-molecular-weight PI3K-family kinase has emerged as a master regulator of DNA damage signaling and extensive cross-talk between ATM and downstream proteins forms an interlaced signaling network. There is rapidly growing scientific evidence emphasizing newly emerging paradigms in ATM biology. In this review, we provide latest information regarding how oxidative stress induced activation of ATM can be utilized as a therapeutic target in different cancer cell lines and in xenografted mice. Moreover, crosstalk between autophagy and ATM is also discussed with focus on how autophagy inhibition induces apoptosis in cancer cells.

Keywords: Ataxia-telangiectasia mutated kinase - signalling landscape - cancer therapy

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Introduction

Research over decades has gradually and sequentially provided near complete resolution of DNA damage signaling and it is now known that DNA damage repair pathway consists of protein network that is highly branched, tightly regulated and well orchestrated. Ataxia-telangiectasia mutated kinase (ATM), ATR and DNA-PKcs are members of phosphoinositide-3-kinase-related protein kinase (PIKK) family. It is evident that DNA damage mediated activation of PIKK family members ATM, ATR and DNA-PKcs is further systematically categorized into modes of repair including homologous recombination (HR) and non-Homologous End Joining (NHEJ).

Emerging lines of evidence reveal that ATM is a master regulator of DNA damage signaling and gate-keeper of genome integrity. It has lately been shown that ATM-mediated mitotic arrest-deficient protein 1 (Mad1) phosphorylation at Serine 214 induced homodimerization of Mad1 and its heterodimerization with Mad2. These mechanisms were noted to be necessary for chromosomal stability (Yang et al., 2014). Hepatocyte nuclear factor-1 alpha (HNF1α) is also phosphorylated by ATM at Ser249 (Zhao et al., 2014). Phosphorylation at serine 403 of HDMX has been shown to facilitate binding of C-terminal RING domain of HDMX to the nascent p53 mRNA to trigger p53 synthesis (Malbert-Colas et al., 2014). Increasingly it is being realized that adhesion between cancer cells and extracellular matrix (ECM) proteins, is an initial step of metastasis. In-vitro evidence has shown that surface expression of α5β1 integrin was increased in the cells treated with ionizing radiation. In MDA-MB-231 cells, it has been reported that α5β1 integrin surface expression was considerably reduced upon ATM inhibition (Lee et al., 2014). Surprisingly it has been shown that caspase-3 processed Cdc6 induced activation of ATM in HeLa cells (Liu et al., 2014). SETD2 is a histone methyltransferase reported to be involved in activation of ATM. In-vitro analysis indicated that SETD2 silenced cells did not show activated ATM kinase (Carvalho et al., 2014). There is an intriguing piece of experimental evidence suggesting that recombinant lysyl oxidase propeptide rLOX-PP considerably reduced radiation induced ATM activation in DU145 and PC3 cells. Likewise, rLOX-PP significantly reduced tumor growth in mice xenografted with cancer cells (Bais et al., 2014). ATM has been shown to phosphorylate deleted in breast cancer-1 (DBC-1) to increase its binding affinity with deacetylase SIRT1. DBC-1 bound SIRT1 was unable to deacetylate p53 thus p53 triggered expression of its target genes. Protein expression of SIRT1 is overexpressed in

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FLT3-ITD+-cells and regulated by FLT3 kinase activity. Genetic or chemical inhibition of SIRT1 remarkably enhanced p53 mediated apoptosis (Sasca et al., 2014). It has recently been persuasively revealed that ATM was not induced in irradiated HER2 silenced MCF-7 breast cancer cells (Yan et al., 2014).

Oxidative Stress Induced Activation of ATM

Elevated levels of ROS and persistent DNA damage are hallmark features of stress induced-premature senescence. Persistent DNA damage is indicative of prolonged ATM activity and wide ranging proteins. Recently emerging evidence is providing an overview of intricate protein network in senescent cells. ATM kinase regulates different proteins in senescent cells. Treating Human endometrium-derived mesenchymal stem cells (hMESCs) with H2O2 induced ROS generation that consequently stimulated ATM kinase activity. Considerably enhanced activity of p38MAPK/MAPKAPK-2 was noted in H2O2 treated cells. Interestingly, there was a persistent DNA damage response and ROS generation noted in H2O2 treated cells. p38MAPK activity modulated prolonged DDR and ROS generation, as cells treated with pharmacological inhibitors of p38MAPK displayed remarkably reduced ROS generation (Borodkina et al., 2014). Moreover, Insulin like Growth Factor (IGF-1) induced intracellular signaling stimulated expression of Secretory clusterin (sCLU) via utilizing MAPK/ERK-1/2/Egr-1 pathway. Functionally inactive ATM expressing fibroblasts have lower expression of sCLU, thus suggesting that ATM also regulates its expression (Luo et al., 2014). Chaetocin, a histone methyltransferase inhibitor has also been noted to trigger ROS mediated ATM activation in glioma cells. Moreover, ATM was involved in induction of apoptosis in glioma cells via different mechanisms. ATM considerably enhanced interaction of p73, p300 and Yes-associated protein 1 (YAP1) in glioma cells. Additionally ATM functionalized intrinsic pathway via activation of Caspase-9. Conversely ATM mediated Caspase-9 activation was impaired upon treatment with ATM inhibitor (Dixit et al., 2014).

Resveratrol Mediated ATM Activation

Certain derivatives of BMH-21, a heterocyclic small molecule triggered DSB induced ATM activation (Colis et al., 2014). Previous studies have shown that Resveratrol induced activation of ATM via DSBs or enhancing generation of ROS. However, it has recently been shown that treating cells with DNA damaging agents or H_2O_2 induced ATM activation, however, these responses were attenuated upon treatment with ATM inhibitors. It was reported that Resveratrol mediated activation of ATM through oxidation. As it is already known that there is a formation of disulfide bonds between ATM monomers, therefore, disulfide-specific reducing agent TCEP was used to note if Resveratrol mediated activation of ATM was impaired. The results revealed notably reduced Resveratrol induced ATM activation in TCEP treated cells (Lee et al., 2014).

Autophagy: Introduction

Autophagy is a biologically complex cytoprotective mechanism. There is an overwhelmingly increasing list of research emphasizing on molecular machinery involved in induction of autophagy. It has been revealed that Atg8 is small ubiquitin (Ub)-like protein noted to be conjugated to autophagic membranes. Atg8 also conjugates different other Ub-like proteins including Atg12-Atg5 to membranes by interacting with Atg12 through its Atg8-interacting motif (AIM). Atg1, Atg13 and Atg17 assembled to form functionally active Atg1. Vesicle nucleation is an intricate step triggered by Atg6 and Vps34 and Atg7 and Atg3 modulate vesicle elongation. Autophagy is a deeply studied mechanism as a response to chemotherapeutic treatment in cancer cells (Marino et al., 2014; Pandey and Chandravati, 2012).

Synthetic and Natural agents mediated regulation of autophagy

Earlier it has been reported that calpain processed Atg5 acted as a pro-apoptotic mitochondrion-permeabilizing fragment. Using papain-like cysteine protease inhibitor (E64d) it was noted that calpain-mediated Atg5 cleavage was remarkably repressed. Atg5 cleavage was also not observed in calpain silenced cells (Yousefi et al., 2006). There is a direct piece of evidence suggesting role of Trichokonin, an antimicrobial peptide in modulating autophagy via calpain-mediated Bax and Atg5 cleavage that induced release of cytochrome c (Shi et al., 2013). Interestingly, Trichokonin triggered ROS generation that triggered disposal of damaged mitochondria within autophagosomes via Atg5-mediated and mitochondria-selective autophagy (Shi et al., 2013). Arnica Montana and Arnica chamissonis derived helenalin has been shown to exert its effects via inhibition of NF-ϰB p65 in MCF-7. Overexpressing p65 in MCF-7 dramatically abrogated helenalin induced autophagy (Lim et al., 2012).

Interplay of ATM and Autophagy

LY2603618 is a Chk1 inhibitor reported to be an inducer of DDR in lung cancer cells. There was an increase in pATM levels in LY2603618 treated lung cancer cells. Surprisingly, autophagy was also induced in LY2603618 treated cancer cells and LY2603618 mediated inhibitory effects on cell proliferation were further enhanced upon treating cancer cells with autophagy inhibitors (Wang et al., 2014). It is also important to mention that autophagy was induced in colorectal cancer cells treated with low dose of camptothecin. Moreover, ATM-Chk2-p53-p21 pathway was also activated in cancer cells that resulted in induction of premature senescence. Autophagy inhibitors considerably enhanced apoptosis in camptothecin treated cancer cells (Zhang et al., 2014). Likewise, Cisplatin induced autophagy in A549 cells as evidenced by higher pATM levels and pAMPK in drug treated cancer cells. Treating cancer cells with ATM inhibitors and autophagy inhibitors resulted in radiosensitization of A549 cells (Toulany et al., 2014). There is contemporary evidence

indicating that ATM triggered activation of AMPK-ULK1 in U87MG and U251 glioma cell lines. Temozolomide induced apoptosis in glioma cell lines treated with ATM inhibitors and autophagy inhibitors (Zou et al., 2014). ATM inhibition with KU55933 induced autophagy in head and neck cancer cells. However, treating cancer cells with autophagy inducers dramatically enhanced KU55933 induced apoptosis (Lin et al., 2012).

It is now known that LKB1/AMPK/mTOR signaling axis controls autophagic induction (Han et al., 2013).

There is an exciting piece of evidence emphasizing on ATM mediated suppression of a negative regulator of autophagy, mTORC1. ATM involvement as an autophagy inducer was further confirmed in ATM-deficient MEFs, in which Nitric Oxide mediated autophagy induction was considerably impaired. ATM activated LKB via its phosphorylation and consequently, AMPK was activated by LKB. This pathway was verified in LKB1-deficient HeLa cells in which ATM was unable to transduce the signals to downstream AMPK because of absence of LKB (Tripathi et al., 2013).

Chemotherapeutic drugs mediated autophagic response in cancer cells is a stumbling block that needs to be overcome. Treating gastric cancer cell line SGC7901 with autophagy inhibitor, significantly enhanced cisplatin induced apoptosis (Zhang et al., 2013). Likewise, treating U251 and U87MG glioblastoma cells with autophagy inhibitors substantially enhanced gambogic acid induced apoptosis (Luo et al., 2012).

Natural Agents Mediated ATM Activation

G19, a sulfated oligosaccharide, prepared from Grateloupia filicina induced oxidative stress mediated activation of ATM in U-87 MG human glioma cells. More importantly, G19 was noted to effectively inhibit tumor growth in mice xenografted with U-87 MG tumor cells (Liu et al., 2014). Riccardin D, a macrocyclic bisbibenzyl, exerts its biological effects via inhibition of telomerase activity in MCF-7 and MDA-MB-231 breast cancer cells. Inhibition of telomerase activity has been shown to activate ATM, that consequently results in induction of apoptosis (Sun et al., 2014). Bufalin, an essential ingredient of Chinese Medicine also induced ATM expression in NCI-H460 cells (Wu et al., 2014). Ampelopsis grossedentata derived Dihydromyricetin is a flavonoid reported to induce DNA damage signaling modulator ATM in osteosarcoma cells. Moreover, p38MAPK and AMPK were also noted to be activated in Dihydromyricetin treated cells. More importantly, Dihydromyricetin exerted its effects via AMPK mediated negative regulation of GSK-3β in osteosarcoma cells (Zhao et al., 2014). Pseudolarix kaempferi Gordon derived Pseudolaric acid B is a diterpene acid recently been shown to induce G2/M arrest in HeLa cells via ATM mediated downstream signaling.

Conclusion

Overwhelmingly increasing list of natural and synthetic agents is currently being tested for efficacy as evidencd by in-vitro and in-vivo analysis. Methanolic extracts of Colchicum baytopiorum were reported to be effective against leukemic cell lines (Pirildar et al., 2010). Bioactive ingredients of Colchicum baytopiorum can further be tested to note if DNA damage signaling modulators are activated in treated cancer cell lines. Research over the years has added very important pieces of information to puzzle of DNA damage signaling and apoptosis. There is a progressive increase in the scientific evidence, deepening our understanding about crosstalk between ATM and autophagy and how autophagy inhibition improves synthetic and natural agents induced apoptosis in cancer cells. ATM is a master-regulator of wide ranging molecular mechanisms and improved knowledge regarding ATM biology will be helpful in maximizing therapeutic values of phytochemicals. Phytochemicals mediated regulation of DNA damage signaling is also providing detailed information about the complex molecular architecture. Phytochemicals mediated control of autophagic response and inhibitory effects on cancer progression are also being studied in xenografted mice. Low bioavailability of phytochemicals is another stumbling block that requires detailed research and it needs to be determined which strategy will be used (intravenous administration or oral intake) for evaluation of efficacy of phytochemicals in cancer models.

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