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Defects in the ATR-Dependent DNA Damage Response Pathway and Human Syndromes

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A multitude of clinically distinct human disorders exist whose underlying cause is a defect in the response to or repair of DNA damage. The clinical spectrum of these conditions provides evidence for the role of the DNA damage response (DDR) in mediating diverse processes such as genomic stability, immune system function and normal human development. Cell lines from these disorders provide a valuable resource to help dissect the consequences of compromised DDR at the molecular level. Ataxia telangiectasia and Rad3-related (ATR) and Ataxia telangiectasia Mutated (ATM) are apical protein kinases that play central roles in coordinating the cell's response to DNA damage. Whilst ATM is activated by DNA double strand breaks (DSB's), ATR is activated by single stranded regions of DNA (ssDNA) which can occur, for example, during DNA replication fork stalling. There is significant functional overlap between these two kinases. In fact, they phosphorylate many of the same substrates, including p53 and Brc1. Nevertheless, ATR appears to be essential for embryonic development, unlike ATM. Mutations in ATM result in Ataxia telangiectasia (A-T) a progressive neurological disorder. Interestingly, a hypomorphic mutation in ATR is associated with Seckel syndrome, a clinically distinct disorder to that of A-T. Seckel syndrome is characterised by profound proportionate growth retardation with severe microcephaly. Why defects in these two related kinases should result in such distinct human disorders is unclear. Recently, mutations in Pericentrin/Kendrin (PCNT) have also been demonstrated in Seckel syndrome. PCNT encodes a core structural centrosomal protein. Interestingly, defective PCNT results in impaired ATR-dependent, but not ATM-dependent G2-M cell cycle checkpoint arrest. Using evidence from murine knockout studies and human cell-based work I will discuss the biological impact of compromised ATR-pathway function with the aim of trying to understand the link between genotype-phenotype in this context.

Keywords: ATR; DNA damage; Human syndrome
exonucleolytic resection generating single stranded overhangs hence shifting the emphasis from ATM signaling to that of ATR. Conversely, stalled replication forks with extensive regions of ATR-activating single stranded DNA can collapse resulting in DSB formation and consequently ATM activation (Figure 1). ATM is recruited to DSB’s via a sensor complex composed of Mre11-Rad50-Nbs1 (MRN complex). Structural studies suggest that this ‘sensor’ complex can tether both ends of the DSB keeping them in close proximity. Biochemical work has also shown that the MRN complex may function to process these ends to ultimately facilitate their repair. An early detectable response to DSB’s is phosphorylation of the histone H2A variant, H2AX, which can be carried out redundantly by ATM or another PI3KK family member, DNA-PK. H2AX phosphorylation (termed γ-H2AX) occurs over megabase pair regions producing discrete, microscopically detectable foci. Formation of these foci is required for the retention of mediator proteins, including 53BP1 and MDC1, which are required for optimal signal transduction response. The principal effector of ATM is Chk2, a kinase that plays a role in controlling the activity of the Cdk25 phosphatase family that are involved in the activation of various Cdk-cyclin complexes. Following DSB-induction, activated ATM phosphorylates and activates Chk2 which subsequently phosphorylates Cdk25A thereby targeting it for rapid ubiquitin-mediated degradation. Consequently, Cdc25A is not available to remove the inhibitory phosphates on Cdk-Cyclin complexes including Cdk1-Cyclin B and Cdk2-Cyclin E. Therefore, these complexes remain inactive resulting in cell cycle arrest, in this case at the G2-M and G1-S/intra-S boundaries respectively. ATM activity can also negatively impact on cell cycle progression following DNA in other ways. Following DSB formation ATM can phosphorylate and stabilise p53. One of its main transcriptional targets is the Cdk-inhibitor p21WAF1/CIP1. Hence active Cdk-cyclin complexes can be rapidly inhibited in response to DNA damage.

ATR exists as a heterodimer with ATRIP (ATR interacting protein) and is recruited to ssDNA via the Replication Protein A heterotrimer (RPA1-3) which rapidly coats single stranded DNA when it occurs within a cell. TopBP1 appears to be required for optimal ATR kinase activity. The MRN complex, along with the Rad17/Rfc2-5 and Rad9/Rad1/Hus1 complexes, are all recruited to the site of damage independently of ATR/ATRIP and are also ATR substrates. Retention of these complexes facilitates ATR’s ability to phosphorylate downstream substrates such as p53. ATR’s key effector is the Chk1 kinase, which coordinates similar and often overlapping signal transduction processes to that of Chk2. Nevertheless, whilst ATM and ATR control overlapping DNA damage response pathways, ATR, unlike ATM appears to be essential for embryonic development and somatic growth. Furthermore, mutations in ATM and ATR confer clinically distinct human conditions. Mutations in ATM cause Ataxia telangiectasia whilst mutation in ATR cause Seckel syndrome (Table 1).

**Ataxia telangiectasia**

Ataxia telangiectasia (A-T) is a progressive neurological disorder characterised by progressive ataxia (loss of muscle coordination), oculomotor apraxia (failure to execute a learned or skilled motor act, in this case eye coordination), dysarthria (impaired articulation-speech impediment), isolated immunoglobulin deficiencies and lymphoma predisposition. A-T is the ‘classical’ cell cycle checkpoint defective disorder as cells from these patients exhibit defects in G1-S, intra-S and G2-M cell cycle checkpoints in response to DSB’s. In fact, failure of intra-S phase checkpoint after IR is used as a diagnostic
tool for this condition (RDS; Radio-resistant DNA Synthesis). A-T cells are also defective in the repair of a subset of DSB’s. Loss of ATM increases genomic instability particularly in response to DSB’s. In fact, lymphoid cells from A-T patients frequently exhibit a specific translocation between chromosome 7 and 14 which arise from a failure to appropriately deal with Recombination Activating Gene (RAG)-induced DSB’s induced at the T cell receptor and immunoglobulin gene loci during V(D)J recombination. ATM knockout mouse models are viable and recapitulate most of the clinical features of A-T (aside from overt ataxia), including tumour predisposition. A recent review on A-T and ATM signalling by Martin Lavin contains a more detailed description of this topic.20

Seckel syndrome

Helmut Seckel first described the syndrome that now bears his name in a monograph published in 1960 entitled “Bird Headed Dwarfs. Studies in Developmental Anthropology Including Human Proportions”. He defined this disorder on the basis of 13 cases from the literature and two personally examined individuals. Seckel syndrome is clinically characterised by severe intrauterine and post-natal growth retardation, profound microcephaly, a ‘bird-like’ facial profile with receding forehead and chin, ‘beak-like’ protruding nose, mental retardation and isolated skeletal abnormalities (e.g. delayed ossification, joint laxity and dislocation). Estimation of the frequency of this disorder is difficult as it has previously been frequently mis- and over-diagnosed.21,22 Nevertheless, the amount of published literature on this disorder suggests that this is a rare condition.

Seckel syndrome is an autosomal recessive disorder and several homozygosity mapping studies indicate that this disorder is genetically heterogeneous (Table 2). The first reported mapping study on Seckel syndrome was by Goodship et al who identified a susceptibility locus on chromosome 3q22.1-q24.23 Subsequently other groups have identified susceptibility loci on chromosome 18 (p11.31-q11.2), chromosome 14 (q21-q22) and chromosome 21 (q22.3) (Table 2).24-26 The first genetic defect identified in Seckel syndrome was a single synonymous mutation in ATR (A>G 2101) in two related Pakistani families originally described in the Goodship et al mapping study (ATR-Seckel syndrome; ATR-SS).27 This mutation caused aberrant splicing of ATR profoundly decreasing ATR expression and disrupting ATR-dependent DNA damage signaling.27 Crucially, it was also shown that this mutation was hypomorphic which was important since it was known that complete knockout of ATR in mice resulted in early embryonic lethality. ATR-SS cells have proved a very useful model tool to study the cellular consequences of a clinically relevant deficiency in ATR-function. ATR-SS cells were found to exhibit increased sensitivity to DNA replication fork stalling (‘nuclear fragmentation’), increased replication fork instability, defective G2-M arrest specifically following UV-irradiation and supernumerary replication fork terminations.28

Table 1.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Gene</th>
<th>Microcephaly</th>
<th>Growth delay</th>
<th>Immunity</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia</td>
<td>ATM</td>
<td>No</td>
<td>Mild, post-natally. Linked to feeding difficulties</td>
<td>Reduced IgA &amp; IgG4. Impaired lymphocyte proliferation, Ig and TCR loci re-arrangements</td>
<td>Non-Hodgkin’s B cell lymphoma &amp; T cell leukaemia</td>
</tr>
<tr>
<td>Seckel syndrome</td>
<td>ATR, PCNT</td>
<td>Yes, Severe.</td>
<td>Severe intra-uterine &amp; post-natal delay</td>
<td>Normal</td>
<td>Not evident, to date.</td>
</tr>
</tbody>
</table>

Table 2.

<table>
<thead>
<tr>
<th>Mapping study</th>
<th>Locus identified</th>
<th>Causative gene identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goodship et al Am J Hum Genet, 2000</td>
<td>3q22.1-q24</td>
<td>ATR</td>
</tr>
<tr>
<td>Borglum et al Europ J Hum Genet, 2001</td>
<td>18p11.31-q11.2</td>
<td>Not yet identified</td>
</tr>
<tr>
<td>Fairve et al Am J Med Genet 2002</td>
<td>Ruled out mapping to chromosomes 3 and 18 thus implying other loci/locus.</td>
<td>None identified</td>
</tr>
<tr>
<td>Killnic et al Europ J Hum Genet, 2003</td>
<td>14q23</td>
<td>Not yet identified</td>
</tr>
<tr>
<td>Griffith et al Nat Genet, 2008</td>
<td>21q22.3</td>
<td>PCNT</td>
</tr>
</tbody>
</table>
centrosomes following treatment with nocodazole in a subset of mitotic cells. This latter phenotype is particularly interesting since centrosome function is known to be essential for normal brain development by mediating a correct balance between symmetric and asymmetric division in the neuroepithelial stem cell layer. Severe microcephaly, a clinical term denoting reduced head circumference, reflects an underlying reduction in brain volume. Whilst microcephaly has multiple aetiologies, both genetic (syndromic) and environmental (e.g. perinatal asphyxia, intrauterine infection) it appears particularly severe in and indeed is a prerequisite diagnostic feature of Seckel syndrome.23

Recently, several distinct mutations in Pericentrin/Kendrin (PCNT), a gene encoding a large protein that plays a structural role in centrosomes, have been identified in several Seckel syndrome families (PCNT-Seckel syndrome; PCNT-SS) (26). PCNT-SS cells showed impaired centrosomal localization of PCNT although interestingly several other centrosomal components investigated localized normally (e.g. centrin, ninnen). Interestingly, PCNT-SS cells also exhibit impaired ATR-pathway function including defective G2-M checkpoint activation and also nocodazole-induced supernumerary mitotic centrosomes.29 This exciting discovery provides the first example of a defect in a structural centrosomal protein impacting on the DNA damage response (DDR) and also for a such defect being associated with a human disorder. Many important events of the cell cycle and DDR originate from the centrosome. For example, the mitotic promoting factor Cdk1-cyclin B is activated initially at the centrosome and Chk1 localisation at the centrosome has been shown to be important for its ability to arrest cells following DNA damage.16,21 How PCNT impacts specifically on ATR-pathway function and not the ATM-pathway remains unclear. Nevertheless, the identification of a defect in a core structural centrosomal protein impacting upon the DDR suggests that defects in other centrosomal components or centrosomally associated proteins could also adversely impact on the DDR. In support of this a recent study has indicated that Cep164, centrosomal protein of unknown function, is required for the maintenance of genomic stability via an MDC1, RPA and a Chk1-dependent mechanism.30

Mutations in PCNT have subsequently been found in the primordial dwarfism disorder Microcephalic Osteodysplastic Primordial Dwarfism type II (MOPDii).31,32 Whilst MOPDii is superficially clinically similar to Seckel syndrome, they are commonly regarded by clinical geneticists as being distinct disorders, thereby implying that they have distinct underlying aetiologies. The principal clinically apparent distinctions between MOPDii and Seckel syndrome appear to be the degree of skeletal involvement (i.e. greater in MOPDii compared to Seckel syndrome), the disproportionate shortness of the forearms and legs in MOPDii and not Seckel syndrome and the fact that microcephaly in MOPDii compared to body size appears to be progressive post-natally, again unlike Seckel syndrome. Nevertheless, MOPDii and PCNT-SS are allelic and PCNT-SS cell lines exhibit and PCNT siRNA knockdown results in compromised ATR-dependent checkpoint activation.33 Whether, Seckel syndrome and MOPDii are distinct disorders or variants of the same underlying genetic defect remains to be unequivocally demonstrated. Nevertheless, there is precedent for clinically defined skeletal disorders subsequently being found to share a common genetic aetiology. Achondrogenesis type B, Diastrophic Dysplasia, Atelosteogenesis 2 and Multiple Epiphyseal Dysplasia are all caused by distinct mutations in the DTDST (Diastrophic Dysplasia Sulphate Transporter) sulphate transporter gene.34 This issue is discussed in more detail in a recent review by Kerzendorfer and O’Driscoll (DNA Repair 2009, In Press).

The link between ATR function and clinical phenotype

Considering the functional overlap between ATM and ATR, an obvious question is why does deficiency of ATR-pathway function result in such a disorder as Seckel syndrome which exhibits major clinical distinctions to that of A-T? Recent work on a conditional knockout murine model for ATR has provided some important leads. Ruzankina and colleagues cleverly circumvented the problem of early lethality during development by mediating a correct balance between symmetric and asymmetric division in the neuroepithelial stem cell layer. Severe microcephaly, a clinical term denoting reduced head circumference, reflects an underlying reduction in brain volume. Whilst microcephaly has multiple aetiologies, both genetic (syndromic) and environmental (e.g. perinatal asphyxia, intrauterine infection) it appears particularly severe in and indeed is a prerequisite diagnostic feature of Seckel syndrome.23

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associated with massive embryonic apoptosis. Unfortunately, this early embryonic lethality rendered the amount of functional information obtainable from this approach somewhat limited. One interesting observation from these studies was that the heterozygote (ATR+) animals were not born at expected Mendelian frequency. This suggested that a diploid complement of ATR was required for normal development. Evidence for an impact of ATR haploinsufficiency in humans is illustrated by a group of conditions referred to as Genomic Disorders.

**Gene copy number variation, Genomic Disorders and ATR**

Gene copy number variation (CNV) appears to be a common genetic trait in clinically unaffected or 'normal' individuals highlighting the plasticity of the human genome. Nevertheless, an expanding list of human conditions known as Genomic Disorders exit representing a clinically diverse group of disorders caused by gain, loss or re-orientation (e.g. inversions) of a genomic region containing dosage-sensitive genes. One class of Genomic Disorder is caused by hemizygous deletions resulting in haploinsufficiency of a single or, more usually, several genes. For example, DiGeorge syndrome is caused by a heterozygous contiguous gene deletion of 1.5-3MBp on chromosome 22q11.2 involving up to about 30 genes. There is mounting evidence, particularly from mouse studies, that haploinsufficiency of several genes whose products encode players in either DNA damage signalling, repair and cell cycle checkpoint activation adversely impacts the normal response to DNA damage and consequently, upon genomic stability (Reviewed in Kerzendorfer and O'Driscoll DNA Repair 2009 In Press). Recently, defective ATR-pathway function has been described in a group of distinct Genomic Disorders involving haploinsufficiency of ATR itself or ATR-pathway components (RPA1, RFC2) (Table 3). The clinical spectrum of these Genomic Disorders includes microcephaly and growth retardation which may indicate that defective ATR-pathway could be a contributing factor to these conditions.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Genomic deletion</th>
<th>ATR-pathway component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subset of Blephorophimosis, Posis Epicanthus, Inversus (BPES)</td>
<td>3q23</td>
<td>ATR</td>
</tr>
<tr>
<td>Miller-Dekker Lissencephaly Syndrome</td>
<td>17p13.3</td>
<td>RPA1</td>
</tr>
<tr>
<td>Williams-Beuren Syndrome</td>
<td>7q11.23</td>
<td>RFC2</td>
</tr>
</tbody>
</table>

**Table 3.**

Monogenic human disorders exhibiting impaired ATR-pathway function

Impaired ATR-pathway function appears to be a feature of cell lines from patients with several distinct DDR-defective conditions. Whilst these disorders are all characterised by a distinct set of clinical features there is significant clinical overlap with Seckel syndrome, particularly concerning the developmental abnormalities such as microcephaly and growth retardation.

**MCPH1-dependent Primary Microcephaly**

Autosomal recessive Primary Microcephaly, a disorder characterised by the presence of a severe microcephaly in the absence of other overt clinical features, is a genetically heterogeneous condition. To date, mutations in four genes, all of which encode centrosomal proteins, have been described for this disorder (MCPH1, ASPM, CDK5/RAP2, CEPNI). Microcephalin (MCPH1), the first Primary Microcephaly gene identified encodes a BRCT-containing product that has been implicated in the response to DSB's. Interestingly, MCPH1-patient derived cell lines phenocopy those of ATR-SS and are defective in ATR-dependent checkpoint activation and also exhibit supernumerary mitotic centrosomes. Furthermore, MCPH1 was found to interact with Chk1, a substrate and downstream effector of ATR.

**Nijmegen breakage syndrome**

Nijmegen breakage syndrome (NBS) is caused by hypomorphic mutations in the Nbs1 component of the MRN complex. Historically, NBS has been described as an 'A-T-like' disorder as cell lines from both conditions exhibit clinical and cellular radiosensitivity, cell cycle checkpoint defects in response to DSB's and similar forms of genetic instability (i.e. chromosomes 7-14 translocations). But, NBS1 is also an ATR substrate and NBS patient-derived cell lines exhibit microcephaly and growth retardation, cell cycle features associated with Seckel syndrome and not A-T. Similar to MCPH1, NBS patient-derived cell lines have also been shown to be compromised for ATR-dependent checkpoint activation and other aspects of ATR-pathway function. Hence, NBS represents a human disorder that exhibits defects in elements of both ATM and ATR-pathway activity and whose clinical features likely represent a compendium of these defects.

**Fanconi anaemia**

Fanconi anaemia (FA), is a genetically heterogeneous condition characterised by a progressive aplastic anaemia, skeletal abnormalities, microcephaly and lymphoid malignancy. FA is caused by mutations in different genes whose products co-ordinately function in the cellular response to DNA cross-links. Several of these genes encode products that together mediate the monoubiquitination and
activation of the FANC-D2 protein in response to DNA damage. ATR and NBS1 have been shown to be required for this specific modification. In fact, ATR-SS and NBS patient-derived cell lines fail to monoubiquitylate FANC-D2 following treatment with replication fork inhibitors. FANCD2 has been shown to be phosphorylated by ATM and ATR further highlighting the overlap between these DDR pathways.

Xeroderma Pigmentosum (Complementation group A; XP-A)

Xeroderma Pigmentosum (XP) is a genetically heterogeneous condition caused by mutations in various components of the nucleotide excision repair pathway (NER), a complex multifactorial DNA repair pathway which detects and excises ‘bulky’ or helix-distorting lesions (e.g. UV-photoproducts) from DNA. Cells from XP patients are characterised by dramatic UV-sensitivity and increased UV-induced mutagenesis. The primary clinical manifestation of XP is a >1000 fold predisposition to basal cell carcinoma of the skin, specifically on sun exposed areas of the body. Progressive neurological degeneration is also frequently seen in XP although its course is highly variable. This manifests clinically as abnormal gait, sensorineural deafness and the lack of deep tendon reflexes. Structural neurological abnormalities such as microcephaly, cerebellar atrophy and enlarged ventricles are also apparent in some XP complementation groups, particularly in XP-A. Endogenously generated DNA damage is implicated in the progression of neurological impairment in this disorder. It has been postulated that reactive oxygen-induced DNA lesions such as cyclo-deoxyadenine and/or cyclo-deoxyguanine may accumulate in neurons of XP patients leading to progressive degeneration.

Interestingly, primary fibroblasts derived from XP-A patients fail to undergo ATR-dependent γ-H2AX formation following UV-irradiation. XP-A cells do not excise UV-photoproducts from their DNA. Since single stranded DNA, an intermediate generated during normal NER, is required for ATR activation, it is likely that the failure to excise UV-photoproducts from the DNA of XP-A results in the consequent failure to activate ATR appropriately. Furthermore, XP-A cells also appear to be defective in the ATR-dependent G2-M cell cycle checkpoint following UV-irradiation. Hence XP-A is characterised by a DNA repair and specific (ATR-dependent) cell cycle defect. It is unclear to what extent, if any, compromised ATR-function could influence the clinical phenotype of XP-A. Although, it is worth noting that microcephaly is a prominent feature this XP complementation group.

Mosaic Varigated Aneuploidy

The mitotic spindle assembly checkpoint (SAC) is a highly complex ordered process that functions to delay the onset of anaphase until all chromosomes are aligned correctly on the metaphase plate thus ensuring accurate segregation of chromosomes into daughter cells. Mosaic Varigated Aneuploidy (MVA) is a congenital disorder of SAC dysfunction. MVA is an autosomal recessive condition characterised by intra-uterine and post-natal growth retardation, microcephaly and an increased frequency of childhood tumours including leukaemia, Wilms’ tumour and rhabdomyosarcoma. Some of these clinical features are reminiscent of Seckel syndrome (ATR-SS, PCNT-SS). To date, mutations in BUB1B, which encodes the SAC kinase BubR1, remain the sole genetic defect described in this condition. Cells from patients with BUB1B mutations are insensitive to mitotic arrest induced by SAC activators such as colcemid. All MVA patient-derived lines typically exhibit mosaic or variable aneuploidies involving different chromosomes, mainly trisomies and monosomies, and premature chromatid separations (PCS) consistent with defective SAC. There is increasing evidence of overlap and communication between the SAC and DDR-mediated cell cycle checkpoint machinery. BubR1 have been found to exhibit reduced DNA damage-induced γ-H2AX formation and defective G2/M checkpoint arrest. Furthermore, Chkl, an effector of ATR, has recently been shown to play a role in the SAC by influencing optimal activation of Aurora-B and BubR1 kinase activities. Whilst a primary defect in ATR-pathway function has not been specifically demonstrated in MVA, the above findings would suggest that it could be a possibility. Furthermore, a phenotypic and functional comparison between MVA and PCNT-mutated MOPDi has been made.

In conclusion

ATR and ATM play pivotal roles in orchestrating the cellular response to DNA damage. Congenital defects in ATR-pathway function are associated with Seckel syndrome in humans. Genetic evidence suggests that further novel Seckel syndrome causative genes await identification. Evidence derived from Seckel syndrome patient cell lines suggests that these novel genetic defects are likely to impair ATR-pathway function. The ATR-pathway appears to be sensitive to haploinsufficiency and several Genomic Disorders that are genetically haploinsufficient for ATR or specific pathway components (e.g. RPAl, RFC2) exhibit defective ATR-function at the cellular level. Furthermore, compromised ATR-pathway function is also a feature of several other DDR-defective conditions, all of which bear some clinical similarity to Seckel syndrome. It is hoped that work using this material coupled with conditional ATR knockdown systems, perhaps even tissue-specific systems, will provide further insight into the mechanisms underlying the cellular and organismal consequences of compromised ATR-pathway function. This should facilitate a greater understanding of why defective ATR-function in humans results in a disorder that is clinically distinct to that of defective ATM (i.e. Ataxia Telangiectasia), perhaps highlighting a hitherto under-appreciated functional link between cell cycle or DNA damage response pathway components and those of tissue-specific developmental pathways.
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References

37. Xu X, Lee J, Stern DF. Microcephalin is a DNA Damage Response Protein Involved in Regulation of CHK1 and BRCA1. *J Biol Chem* 279: 34091-34094, 2004
48. Musacchio A, Salmon ED. The spindle-assembly checkpoint in space and time.


