Artemis, a novel guardian of the genome*

Minireview

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B and T lymphocytes recognize foreign antigen through specialized receptors: the immunoglobulins and the T cell receptors, respectively. The highly polymorphic antigen-recognition regions of these receptors are composed of variable (V), diversity (D), and joining (J) gene segments that undergo somatic rearrangement prior to their expression by the V(D)J recombination process. Proper joining of the V, D, and J segments requires the participation of the Rag proteins as well as the non-homologous end-joining (NHEJ) factors. Recently, a novel V(D)J recombination/NHEJ factor, Artemis, has been identified. Mutations in the *ARTEMIS* gene cause human severe combined immunodeficiency with increased radiosensitivity (RS-SCID), an autosomal recessive disease characterized by the absence of the T and B lymphocytes and by a defect in the V(D)J recombination. This minireview compiles all mutations in the *ARTEMIS* gene identified so far. Furthermore, phenotypes of RS-SCID patients and links to the particular mutations are described. Biochemical and structural properties of the Artemis proteins are reviewed and integrated into the processes of V(D)J recombination and NHEJ. A genomic caretaker function is assigned to Artemis.

Key words: Artemis, V(D)J recombination, non-homologous end-joining, severe combined immunodeficiency with increased radiosensitivity.

DNA double-strand breaks

DNA damaging agents constantly challenge DNA inside cell. Therefore, during the lifetime DNA tends to accumulate lesions. DNA double-strand breaks (DSBs) are one of the most detrimental DNA lesions, as they disrupt both DNA strands, causing problems for DNA transcription, replication and segregation. DSBs can be induced by exogenous agents such as ionizing radiation (IR) or a number of chemicals, as well as by endogenous sources such as free radicals generated during metabolic processes. Moreover, DSBs arise in DNA as intermediates during cellular processes, such as replication, meiotic and mitotic recombination, mating-type switching in yeast, DNA repair (e.g. during the processing of the interstrand cross-links [ICLs]), transposition of mobile elements and V(D)J recombination in mammalian cells [11, 33]. Under certain conditions, even one DSB can kill a cell. If repaired inaccurately or left unrepaired, DSBs can cause gene deletion, chromosome loss and other chromosomal aberrations that increase genetic instability and might ultimately produce cancer diseases such as leukemias, lymphomas, osteosarcomas and others [16, 34].

V(D)J recombination

B and T lymphocytes recognize foreign antigen through specialized receptors: the immunoglobulins and the T cell receptors, respectively. The highly polymorphic antigen-recognition regions of these receptors are composed of variable (V), diversity (D), and joining (J) gene segments that undergo somatic rearrangement prior to their expression by a mechanism known as V(D)J recombination. V(D)J re-

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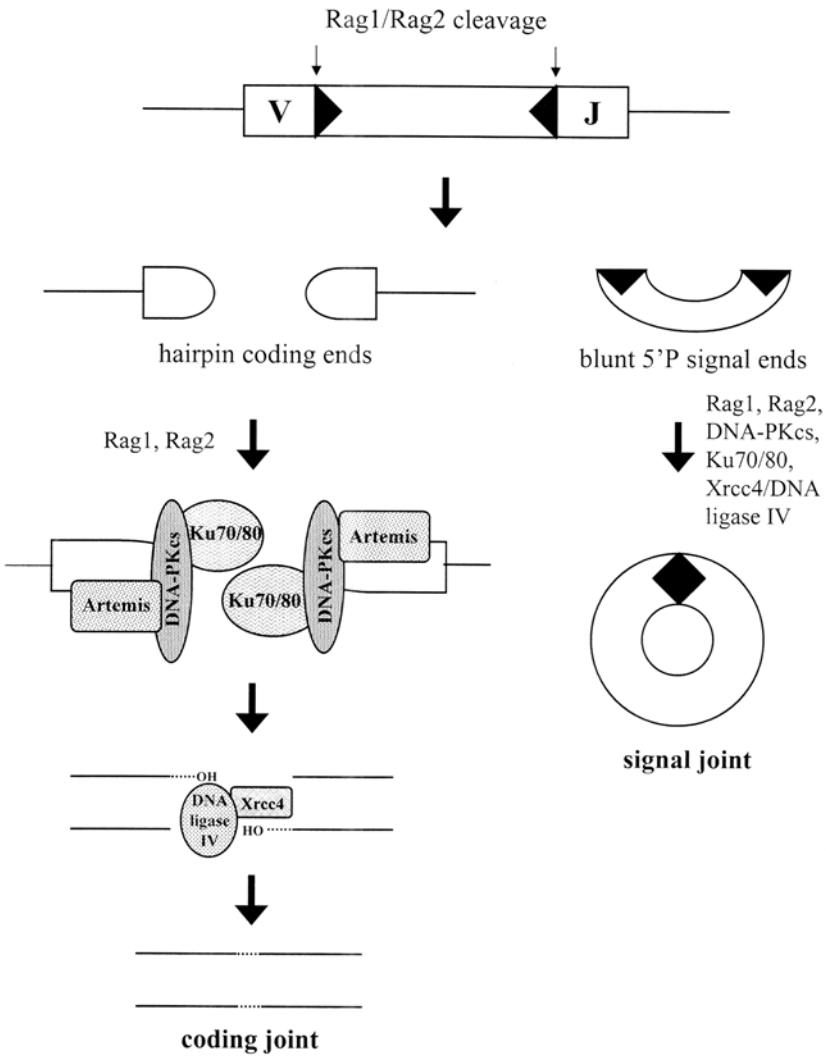

Figure 1. The V(D)J recombination pathway is initiated by binding Rag1/Rag2 proteins to specific recombination signal sequences (RSSs) (the triangles) that flank the immunoglobulin or T cell receptor gene segments (the rectangles, here denoted V and J). The Rag1/Rag2 protein complex cleaves the DNA precisely between the specific RSSs and the coding segments, producing blunt signal ends and covalently sealed hairpinned coding ends. Rag1/Rag2 and the NHEJ proteins are required for forming both coding and signal joints. The signal junctions undergo accurate rejoining. However, hairpinned coding ends must be opened by endonucleolytic cleavage before forming the coding joint. Firstly, Ku70/80 binds to the ends and recruits a complex of DNA-PKcs and Artemis. Artemis, after its association with, and phosphorylation by DNA-PKcs, acquires an endonuclease activity and can open the Rag1/Rag2-generated hairpin structures at the DSB. Further modification and fill-in occurs prior to ligation by the DNA ligase IV/Xrcc4 complex, creating diversity of the coding junctions.

combination is initiated by the Rag1/Rag2 protein complex, encoded by the recombination activating genes (*RAG1* and *RAG2*). The complex recognizes the recombination signal sequences (RSSs) that flank the V, D, and J segments and introduces DSBs between the RSSs and the coding sequences [10]. The cleavage leaves blunt signal ends (with RSSs at the ends) that can be directly ligated, and covalently sealed hairpinned coding ends that require further processing before the rejoining of different segments into various exon-encoding antigen recognition sites (Fig. 1).

Non-homologous end-joining takes part in V(D)J recombination

Proper joining of the V, D, and J segments requires the participation of the Rag proteins as well as the non-homologous end-joining (NHEJ) factors such as a DNA-dependent protein kinase (DNA-PK), DNA ligase IV, Xrcc4 and Artemis [29]. An additional factor might be required for NHEJ and V(D)J recombination as recently shown [8].

NHEJ represents DSB repair mechanism that does not require extensive sequence homology. The original sequence is restored only if the DSB generates two complementary or blunt ends. If, however, two non-matching ends arise, they first have to be transformed into a ligatable structure by enzymatic modifications. Because NHEJ factors repair all sorts of DSBs, and loss of their function causes genomic instability through impaired end-joining, these proteins have been called "genome guardians".

The DNA-PK is a trimeric enzyme composed of the catalytic subunit (DNA-PKcs), a serine/threonine kinase that belongs to the phosphatidylinositol-3-kinase family, and a dimeric component consisting of the Ku70 and Ku80 proteins (Ku70/80). DNA-PK may function as a DNA damage sensor; its DNA break-dependent activation triggers an intracellular signaling pathway through phosphorylation of specific target proteins. Additionally, DNA-PK may act directly in NHEJ by functioning as a scaffold for assembly and coordination of the repair enzymes at the DNA breaks. The signaling and scaffolding functions of DNA-PK may act in concert to facilitate the cellular response to DNA damage and subsequent repair [13]. Ku70/80 is a highly abundant DNA-binding protein [1]. Once bound to DNA, it prevents digestion of the broken ends by DNA exonucleases, recruits DNA-PKcs to DNA ends and triggers its kinase activity towards a variety of DNA-bound proteins in the vicinity. DNA-PK is capable of autophosphorylating Ku70, Ku80 and DNA-PKcs *in vitro*. Autophosphorylation

of DNA-PKcs results in dissociation from Ku70/80 and loss of the kinase activity [5, 6]. Interestingly, DNA-PK phosphorylates also two proteins, which might be required for the processing of subclasses of DSBs, the helicase/exonuclease protein deficient in the human premature ageing Werner syndrome [17, 40] and Artemis [24].

Xrcc4 forms a tight complex with DNA ligase IV (DNA ligase IV/Xrcc4) in a DNA-independent manner [28] and stimulates its activity by increasing the yield of ligase adenylation [20]. Moreover, Xrcc4 interacts weakly with, and is phosphorylated by, DNA-PK *in vitro* [7]. It has been shown that Ku70/80 recruits DNA ligase IV/Xrcc4 to DNA ends *via* protein-protein interactions [26] and that it stimulates DNA ligase IV-dependent ligation by mechanism that requires its movement along the DNA molecule [19].

In humans and mice that harbor mutation in the DNA-PKcs encoding gene, the coding joint formation is severely impaired, although the rejoining of signal ends appears relatively normal. In the striking contrast, mutations in each of the other known NHEJ proteins are associated with defects in both signal and coding joint formation [36]. Xrcc4- and DNA ligase IV-deficient mice exhibit a different pleiotropic phenotype, including late embryonic lethality due to massive apoptosis of newly generated neurons, and various cellular defects.

Mutations that allow joining but cause it to be misregulated, e.g. allowing broken DNA ends to join the wrong partner chromosome, can lead to chromosomal instability and lymphoid malignancies. Hypomorphic mutations in any of these genes could deregulate joining sufficiently to increase the incidence of oncogenic rearrangements. Inherited or acquired mutations in the NHEJ factors could be important risk factors for developing lymphoid neoplasms and perhaps other types of cancer as well [2]. Mutations that block recombination by preventing hairpin opening and thus coding joint formation cause a severe combined immunodeficiency (SCID) of both B and T lymphocytes. There is no proof yet that human SCID cases are associated with mutations in the above-mentioned NHEJ factors [21, 22].

Artemis, a novel V(D)J recombination/NHEJ protein

Two years ago, when studying molecular background of children with SCID and reduced tolerance to radiation, Moshous and colleagues [29] found that the disease was caused by mutations in a particular gene. They called the gene *ARTEMIS*, after the Greek goddess, the guardian of young children and small animals.

The *ARTEMIS* gene is composed of 14 exons, the size of which ranges from 52 bp to 1160 bp. The gene was mapped to chromosome 10p. Its predictive protein consists of 685 amino acids and has a molecular weight of 77.6 kDa. Human Artemis is 78% identical to its murine counterpart [29]. Artemis shares homology with the yeast Snm1/Pso2 protein that was shown to be involved in repair of DNA damage caused by ICL agents but not IR [12].

The expression of the *ARTEMIS* gene is extremely low presumably due to the presence of an internal ribosome entry site (IRES) in its 5' untranslated region. In fact, one function of the IRES may be to maintain Artemis at low levels since overexpression is highly toxic to mammalian cells and appears to result in apoptosis [9]. This phenomenon may be a characteristic of the *SNM1/PSO2* family members since similar results have been observed with the human *SNM1* and *SNM1B* genes (for details on the human Snm1/Pso2 homologues, see text below) [29].

As mentioned above, *ARTEMIS* codes for a novel V(D)J recombination/NHEJ factor. Artemis belongs to a large superfamily of proteins that adopted the metallo-β-lactamase fold as part of their putative catalytic site. The members of this superfamily are known to function enzymatically in reaction that utilizes water molecules as nucleophiles to break covalent bonds. Actually, Artemis belongs to one branch of this superfamily, which comprises proteins possessing nucleic acid substrates. Metallo-β-lactamase fold consists of a four-layered β-sandwich with two mixed β-sheets flanked by α-helices, with the metal-binding sites located at one edge of the β-sandwich. The binuclear Zn(II) centre, used to perform the cleavage reaction, is located at the bottom of a wide shallow groove [38]. Five sequence motifs, consisting mostly of histidine and aspartic acid residues, are highly conserved in active enzymes of the superfamily and participate in zinc coordination and hydrolysis reaction. Prompted by an interest in the Artemis function in DNA repair, Callebaut and colleagues [4] undertook a detailed analysis of its C-terminal sequence to motif four, up to which the similarity with proteins of the metallo-β-lactamase superfamily can be significantly detected against domain databases. Indeed, motif five has not been identified in Artemis. Instead, this C-terminal sequence of 360 amino acids shares obvious similarity with yeast and mouse Snm1/Pso2 and, to a lower extent, with the 73 kDa subunit of the cleavage and polyadenylation specificity factor (Cpsf) [29]. The conserved sequences lying after the four typical metallo-β-lactamase motifs therefore constitute a hallmark of proteins of this family specifically acting on nucleic acids. They are not restricted to a limited region, the length of which should correspond to that including the metallo-β-lactamase motif five, but share features of a distinct globular domain that was named the β-CASP motif, after metallo-β-lactamase-associated Cpsf Artemis Snm1/Pso2. β-CASP domain, although highly divergent and tolerating multiple insertions, harbors several conserved residues that could target the activity of this subgroup of proteins toward nucleic acids and thus, serves the purpose of DNA repair. Other domains, yet to be defined, are probably responsible for directing the various Artemis/Snm1/Pso2 proteins to their specific DNA re-

pair pathways, DSB repair and ICL repair in case of Artemis and Snm1/Pso2, respectively [4].

Given the structural homology of Artemis to metallo-β-lactamases, Ma and colleagues have tested Artemis for its nuclease activity *in vitro* [24]. The purified Artemis protein alone possesses intrinsic single-strand-specific 5' to 3' exonuclease activity in the presence of Mg^{2+}, but lacks any nuclease activity in the presence of Mn^{2+}. Moreover, Artemis forms a complex with DNA-PKcs in the absence of DNA that is stable under physiologic ionic strength and does not rely on DNA terminations or the presence of Ku70/80 for stability. Furthermore, upon complex formation, DNA-PKcs efficiently phosphorylates Artemis and this activity depends on DNA ends, suggesting that the Artemis/DNA-PKcs nuclease complex would be very easily responsive to pathologic DSBs. Following phosphorylation, Artemis acquires an endonuclease activity and can open the Rag1/Rag2-generated hairpin structures at the DSB as well as 5' and 3' overhanging ends. Since DNA-PKcs alone did not show nuclease activity and a point mutant of Artemis possesses the failure to open hairpins, the nucleolytic active site probably resides in Artemis. Moreover, the fact that the regulation of Artemis endonucleolytic activity by Artemis/DNA-PKcs is ATP-dependent indicates that the kinase activity of DNA-PKcs is necessary. It seems interesting that pre-treatment of Artemis with DNA-PKcs and ATP is not sufficient to confer overhang cleavage and hairpin-opening activity on Artemis. DNA-PKcs must remain present, even after the phosphorylation, for efficient hairpin opening. Thus, DNA-PKcs regulates Artemis by both phosphorylation and complex formation to permit enzymatic activities that are critical for the hairpin-opening step of V(D)J recombination and for 5' and 3' overhang processing in NHEJ [14, 24].

Interestingly, there are specific locations on the hairpin at which Artemis/DNA-PKcs hydrolyzes the phosphodiester bond. When hairpins are opened at positions other than the precise tip, an inverted repeat is generated at the resulting overhang. Such inverted repeats, named palindromic nucleotides, were initially identified in V(D)J recombination coding joints in chickens [25] and subsequently in all vertebrates. Thus, palindromic nucleotides probably arise as a result of opening of hairpin intermediates at nontip positions.

Recently, the positional preferences for the 5' overhang, 3' overhang, and hairpin endonucleolytic activities of Artemis/DNA-PKcs have been explained. On 5' overhangs, the endonucleolytic cleavage preference is directly at the single- to double-strand transition. On 3' overhangs, the endonucleolytic cleavage preference is displaced ~ 4 nucleotides into the single-stranded region. Hence, it appears that Artemis/DNA-PKcs recognizes 4 nucleotides of ssDNA (nearest to a double-strand transition) in an orientation-dependent manner, and it preferentially cleaves at the 3' side of that 4 nucleotides ssDNA region [24].

Artemis may also perform non-nucleolytic functions. The rare T cell receptor rearrangements isolated from patients with hypomorphic Artemis mutations show severely curtailed N region diversity [30]. N regions are nontemplated nucleotides added to the 3' ends of Rag-mediated DSBs by the enzyme terminal deoxynucleotidyl transferase to provide added junctional diversity. Moreover, N region addition requires Ku70/80, probably to recruit other end-processing factors, and Artemis may assist in this recruiting. Nonetheless, Artemis-deficient mice showed no decrease in N regions. These differences between patients and mice (i.e. mice produce some coding joints and add N regions while patients do not) could reflect species-specific differences in the NHEJ process [2].

Similarly, *ARTEMIS* knockout mice present an overall phenotype like human SCID patients and DNA-PKcs-deficient mice, including normal size and cellular proliferation, but not RSS joining and increased cellular sensitivity to IR [35]. Moreover, Rooney et al have shown that impaired T cell development is associated with the accumulation of unresolved hairpin intermediates in *artemis* defective thymocytes, strongly supporting the suggestion that Artemis functions in processing hairpin coding ends. They have also described a spontaneous chromosomal instability in fibroblasts from *ARTEMIS* knockout mice, proving the notion that Artemis is a genomic caretaker [35].

The *ARTEMIS* gene mutation causes SCID

SCID is a group of genetically and phenotypically heterogeneous disorders that is immunologically characterized by the absence or dysfunctioning of T lymphocytes. It can be subdivided based on the additional presence or absence of B lymphocytes and natural killer (NK) cells in peripheral blood. SCID with the T^-B^+ phenotype accounts for ~45% of cases, two-thirds of which are inherited as X-linked, common γ-chain-receptor deficiency. The remaining one-third of T^-B^+ SCID cases has an autosomal recessive inheritance and includes Jak3 protein kinase deficiency and Zap70 deficiency [21].

In the most severe form of SCID, both T and B lymphocytes are absent because of a defect in the V(D)J recombination process, whereas NK cells are normally present ($T^-B^-NK^+$ SCID). Children with SCID have an unusual number of bacterial, viral, fungal, or protozoal infections that are life-threatening and less responsive to treatment than would normally be expected. These include, but are not limited to, pneumonia, meningitis, and/or blood system infection. Without bone marrow transplantation, a child with SCID is at risk for severe or fatal infection and may be best kept in a sterile isolation. Without treatment, SCID is often fatal within the first year of life [29].

About 30% of $T^-B^-NK^+$ SCID patients carry mutations in

Table 1. Characterization of radiosensitive severe combined immunodeficiency patients

Origin	Clinical features	Patient	Status	Mutation	Effect of mutation	Reference
Athabascan-speaking Americans	Early onset of serious infections Failure to thrive Severe oral and/or genital ulcers	P1-20	Homozygote	Nonsense mutation (TAC→TAA) in exon 8	Premature stop codon → truncation of protein	[22]
		P21	Heterozygote	Nonsense mutation (TAC→TAA) in exon 8 and the absence of the maternal allele		
Japanese	Hypogammaglobulinemia Cytomegalovirus infection	P22-23	Homozygote	Genomic exon 3 deletion	Alternative splicing and the loss of adjacent exons; frame shift → premature termination	[18]
		P24	Heterozygote	Genomic exon 3 deletion and AG insertion between 1205T and 1206G in exon 14		
	Bronchiolitis obliterans	P25	Heterozygote	Genomic exon 3 deletion		
Europeans	Candidias Protracted diarrhea Hypogammaglobulinemia Autoimmune anemia Thrombocytopenia Severe lymphocytopenia Recurrent pulmonary and respiratory infections Bronchopneumonia Otitis	P26-28	Homozygote	Genomic exons 1-4 deletion	No RNA	[29]
		P29	Heterozygote	Genomic exons 1-4 deletion and nonsense mutation (C→T) at position 279	No RNA; Arg74 → nonsense codon	
		P30	Homozygote	Nonsense mutation (C→T) at position 279	Arg74 → nonsense codon	
		P31	Heterozygote	Nonsense mutation (C→T) at position 279 and nucleotide change (G →A) in exon 10 splice donor site	Arg74 → nonsense codon; fusion of exons 9-12; deletion of Ala261-Glu317	
		P32	Homozygote	Genomic exons 5-8 deletion	In-frame splicing of exons 4-9; deletion of Lys96-Gln219	
		P33	Heterozygote	Genomic exons 5-6 deletion and nucleotide change (G →C) in exon 11 splice donor site	Out-of-frame splicing of exons 4-7; Lys96 frameshift; Out-of-frame splicing of exons 10-12; Thr300 frameshift	
		P34-37	Homozygote	Nucleotide change (G →T) in exon 5 splice donor site	Out-of-frame splicing of exons 4-6; Lys96 frameshift	
		P38	Homozygote	Nucleotide deletion (G) at position 818 in exon 9 and nucleotide change (C →T) at position 827 in intron	Ala254 frameshift	
		P39-41	Heterozygote	Genomic exons 1-3 deletion and nucleotide deletion (T1384-A1390) in exon 14	Loss-of-function mutation; frameshift → premature stop codon	[30]
		P42	Homozygote	Nucleotide deletion (A1328-T1344) in exon 14	Frameshift → premature stop codon	
		P43	Homozygote	Genomic exons 10-12 deletion	Frameshift → premature stop codon	[32]
		P44	Homozygote	Substitution (G→T) at position 47 in exon 5	Gly → Val at position 111	
		P45-46	Homozygote	Substitution (G→A) at position 42 in exon 6	Gly → Glu at position 128	

either of the *RAG* genes. However, fibroblast cells and bone marrow of some $T^-B^-NK^+$ SCID patients without the *RAG* genes mutations show a remarkably increased sensitivity towards IR, and their fibroblasts also show impaired coding joint formation in an extrachromosomal V(D)J recombination assay. Some of these IR sensitive SCID patients (RS-SCID) have large deletions or truncation mutations in the *ARTEMIS* gene [32].

Although $T^-B^-NK^+$ SCID is very rare in the general population (1–2 per million live births), it has a high incidence (1 per 2000 live births) in groups of American Indians whose main common feature is the Athabascan language. They are members of three tribes, Navajo and Apache in southwest USA and the Dogrib in northwest Canada. In addition, the same phenotype of disease has been identified in three patients of two related families among the Diné Indians, a small tribe of Athabascans living in the Northwest Territories [21]. The SCID in Athabascan language speaking people (SCIDA) is inherited as an autosomal recessive trait, the gene being on chromosome 10p. The SCIDA condition appears quite unique and homogenous; all patients have similar laboratory findings of severe T and B lymphopenia (lymphocyte counts <500, with <5% T and B cells) and very low (<5% of normal) to absent lymphocyte proliferation in response to mitogens and alloantigens. Clinically, it is characterized by the early onset of serious life-threatening infections and the peculiarity of this form seems to be presentation with oral and genital ulcers. The affected children usually die from severe infections within 6 months of age unless treated with successful bone marrow transplantation. Li and colleagues [22] have studied 21 SCIDA patients of Navajo and Apache origin (Tab. 1) and 20 of them carried the homozygous nonsense mutation (TAC→TAA) in exon 8 of the *ARTEMIS* gene, which was not seen in any of the 30 normal controls, while it was carried by their parents. This nonsense mutation creates a premature stop codon and results in a decreased level of *ARTEMIS* transcription and the truncation of the protein product. However, the V(D)J recombination defect found in the fibroblast cells of SCIDA patients can be effectively complemented by their transfection with the wild-type *ARTEMIS* primary transcript [22].

More recently, four Japanese patients have been identified to have RS-SCID attributable to a mutation of the *ARTEMIS* gene (Tab. 1). They are the first cases in East Asia, and surprisingly, all of them are from four unrelated families and come from different parts of Japan. Moreover, they carry two novel mutations of the *ARTEMIS* gene. One novel mutation is a genomic exon 3 deletion, the second is the insertion of two nucleotides (AG) between 1205T and 1206G in exon 14. Both mutations result in a frame shift and premature termination [18]. As the genomic deletion of exon 3 is carried by all four patients (two being homozygous and two heterozygous for this mutation), it appears to be a common mutation for Japanese RS-SCID. Contrary, 13 predominantly RS-SCID patients from 11 unrelated families from Europe (Tab. 1) have different mutations in the *ARTEMIS* gene, including premature stop codons, splice site mutations, nucleotide deletions, and several different genomic deletions, suggesting that the *ARTEMIS* gene may represent a hot spot for a gene deletion [29].

So far, all mutations identified in the *ARTEMIS* gene were complex or nonsense mutations [18]. Very recently, Noordzij and colleagues [32] have identified patients with simple missense mutations (Tab. 1). The two point mutations were in evolutionary highly conserved amino acid residues within the *SNM1/PSO2* homology domain, suggesting that this domain is required for the catalytic activity [32].

The yeast homologue of *ARTEMIS*, the *SNM1/PSO2* gene

The *S. cerevisiae snm1* and *pso2* mutants were isolated as a result of screening for mutants with increased sensitivity to nitrogen mustard (HN2) and 8-methoxypsoralen (8-MOP) plus UVA light, respectively. Later, the genes affected in these mutants were shown to be allelic. *SNM1/PSO2* is the non-essential gene encoding a 76 kDa protein with one putative zinc-finger motif, nuclear transport signal [9, 31] and metallo-β-lactamase domain [24]. *SNM1/PSO2* mRNA contains the IRES in its 5' untranslated region that generally suppresses translation, but specifically upregulates gene expression during mitosis [41]. The constitutive expression of *SNM1/PSO2* is therefore extremely low (approx. 0.3 transcripts/cell), but is inducible through the *DUN1* pathway by treatment with ICL agents, such as HN2, cisplatin, or 8-MOP plus UVA light [39].

While the yeast *snm1/pso2* mutants demonstrate a strong defect in the repair of DNA damage caused by a number of ICL agents, they do not display elevated sensitivity to IR (for a review, see [3, 12]). This is in a sharp contrast to the phenotype of RS-SCID patients whose primary molecular defect is indeed the absence of DSB repair, illustrated by the lack of coding joint formation in the course of V(D)J recombination and the increased sensitivity of bone marrow and fibroblast cells to IR [32].

The *snm1/pso2* mutant cells are normal in forming incisions at sites of ICLs in DNA, and thus, in DSB formation in response to ICL. However, they appear defective in the restoration of high-molecular weight DNA during the latter stages of ICL repair [27]. Surprisingly, the *snm1/pso2* mutants are normal in repair of HO-induced DSBs. This suggests that cells with mutations in the *SNM1/PSO2* gene are not able to repair DSBs formed as an intermediate stage during ICL repair and that the function of the Snm1/Pso2 protein is implicated in a post-incision processing step. Moreover, action of Snm1/Pso2 does not rely on HR, since the *SNM1/PSO2* pathway does not utilize HR for survival

after ICL formation. However, the function of Snm1/Pso2 does depend on the conserved metallo-β-lactamase domain, since disruption of that domain inactivates the gene for ICL repair [23].

The Snm1/Pso2 family

The database search revealed the existence of the Snm1/Pso2 homologues in many different species, including *Schizosaccharomyces pombe*, *Aspergillus niger*, *Arabidopsis thaliana*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Danio rerio*, *Rattus norvegicus* and *Homo sapiens*. In mammals, five homologues of the yeast Snm1/Pso2 protein have been identified so far. These include HsSnm1, HsSnm1B and Artemis (also referred to as HsSnm1C) [9]. Two other more distantly related proteins include a subunit of the Cpsf involved in RNA processing and a novel putative prostate cancer susceptibility protein Elac2 [15, 37]. Interestingly, all of the *SNM1/PSO2* homologues have in common a homologous region that encodes a metallo-β-lactamase domain [29, 41], but are unique outside of this domain, suggesting that each gene have a distinct function.

Conclusions and perspectives

Because the absence of Artemis has been shown to be compatible with sustained human life and cellular survival, Artemis appears to be a strong candidate for a tumor suppressor in humans. This opinion is strengthened by findings that its inactivation causes genomic instability in mice [35]. *In vitro*, Artemis possesses both the endonuclease and exonuclease activities, which seem to be necessary for process of V(D)J recombination and NHEJ. The endonuclease activity is acquired following phosphorylation by DNA-PKcs [24], suggesting an *in vivo* regulation of Artemis's function. It would be therefore interesting to find out conditions under which phosphorylation of Artemis is triggered. Moreover, identification of phosphorylation sites in the Artemis protein and of the responsible protein kinase(s) (DNA-PKcs might not be exclusive protein kinase involved in phosphorylation of Artemis) could lead to better understanding of Artemis-dependent cellular processes. Finally, addressing the question of whether the Artemis's nuclease function might be substituted under certain conditions by other nuclease(s) (for example by the Mre11-Rad50 complex) could further specify the requirement for Artemis in human life.

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