Recent advances in the study of immunodeficiency and DNA damage response

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Abstract DNA breaks can be induced by exogenous stimuli or by endogenous stress, but are also generated during recombination of V, D, and J genes (V(D)J recombination), immunoglobulin class switch recombination (CSR). Among various DNA breaks generated, DNA double strand break (DSB) is the most deleterious one. DNA damage response (DDR) is initiated when DSBs are detected, leading to DNA break repair by non-homologous end joining (NHEJ). The process is critically important for the generation of diversity for foreign antigens; and failure to exert DNA repair leads to immunodeficiency such as severe combined immunodeficiency and hyper-IgM syndrome. In V(D)J recombination, DSBs are induced by RAG1/2; and generated post-cleavage hairpins are resolved by Artemis/DNA-PKcs/KU70/KU80. DDR is initiated by ataxia-telangiectasia mutated as a master regulator together with MRE11/RAD50/NBS1 complex. Finally, DSBs are repaired by NHEJ. The defect of one of the molecules shows various degree of immunodeficiency and radiosensitivity. Upon CSR inducing signal, DSBs induced by activation-induced cytidine deaminase and endonucleases elicit DDR. Broken ends are repaired either by NHEJ or by mismatch repair system. Patients with radiosensitive SCID require hematopoietic cell transplantation as a curative therapy; but the procedures for eradication of recipient hematopoietic cells are often associated with severe toxicity.

Keywords DNA damage response · Immunodeficiency · V(D)J recombination · Class switch recombination

Introduction

DNA damage response (DDR) is critically important in maintenance of proper cell functions. Among various DNA damage, a double strand DNA break (DSB) is the most deleterious which necessitates proper DNA response, resulting in successful cell survival when appropriately repaired or in cell death when the lesions are misrepaired. Inappropriate DSB repair can result in chromosomal aberration or translocation; the survived cells with the lesions then can develop malignancy when acquire growth advantage.

Cells respond to the DNA breaks with a sophisticated DDR, starting from detection of DNA lesions, delivering DNA damage signaling, initiating DNA break repair system, and then deciding the cell fate [1]. The process is executed with participation of multiple molecules in an orchestrated manner. Generated DSBs activate a specific signal cascade that is mainly regulated by the kinase, ataxia-telangiectasia mutated (ATM), and are repaired mainly by non-homologous end joining (NHEJ). Homologous recombination (HR) also functions for the repair of broken ends but does so only in specific occasions when a sister chromatid is present [1–5].

DSB can be induced by exogenous stimuli or by endogenous stress such as replication, but is also generated in cells with a programmed manner. For example, the programmed DSB is observed in lymphocytes development and in meiosis [5, 6].

V(D)J (variable: V, diversity: D, and joining: J) recombination is a DNA rearrangement process that occurs at Immunoglobulin heavy chain (IgH) loci, Immunoglobulin light chain loci (Igk and Igλ), and at T cell receptor loci

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(TCR α , β , γ , δ), and thus is critical in development of B and T lymphocytes. Immunoglobulin class switch recombination (CSR) occurs at IgH constant regions, and replaces C μ to C γ , C α , or to C ϵ , resulting in the production of IgG, IgA, or IgE. RAG1/2 lymphoid specific factors initiate DSB formation in V(D)J recombination; and activation-induced cytidine deaminase (AID) triggers DSB formation in CSR response [5–7].

More than twenty molecules are involved in the V(D)J recombination and CSR; and inability to persecute the programmed DDR results in primary immunodeficiency (PID) (Table 1) [3, 6, 8–15]. The patients with defect either in DNA damage signal or in NHEJ process present PID phenotype, and often show one or more of phenotypes that include radiosensitivity, predisposition to malignancy, developmental delay, and neurological deficits (Table 2). These disorders provide us with an insight into physiological importance and function of each molecule in health and disease, specific function of a domain in each molecule, and with how DDR system works as a complex in a regulated manner.

Since detailed machinery of DDR and impact of defective responses to DNA damage on immune cell development and tumorigenesis have been covered by several well-described papers [3, 6, 8, 10, 12–15], this review will mainly focus on phenotypes of PID due to impaired DDR including newly discovered disorders (Fig. 1).

Molecular event of V(D)J recombination and CSR

In order for immune cells to respond to various foreign antigens, lymphoid cells generate great diversity of antigen receptors, B-cell receptor (BCR) in B cells and T-cell receptor (TCR) in T cells. The receptors exhibit numerous variety through variable domains generated through the combination of two or three gene regions (VDJ or VJ). V, D, and J domains are consisted as multiple copies, thus enabling the production of diversity by the combination of V, D, J or that of V and J. The coding genes, V, D, and J, are flanked by recombination signal sequences (RSS); and V(D)J recombination is initiated by recognition of the RSS and by cleavage at the site, by sequence-specific recombinase, RAG1 and RAG2 [16]. The programmed DNA break and DNA repair are restricted at the G0/G1 phase; and the generated DSB is repaired by NHEJ. When DSBs are induced, coding ends of *BCR* or *TCR* gene loci are sealed by a hairpin. The hairpin is bound by KU70/80 which form DNA dependent protein kinase (DNA-PK) complex together with DNA-PK catalytic subunit (DNA-PKcs coded by *PRKDC* gene), and the complex recruits Artemis (DCLRE1C) [9]. DNA-PK phosphorylates Artemis; and activated Artemis opens the hairpin via its endo-

exonuclease activity. DNA damage signaling molecules also play a role at this programmed DDR. ATM and the MRN complex (MRE11, RAD50, and NBS1 that are stable only when all the components are present) accumulate at the break site and initiate DDR signal [17]. P53 is one of key substrates of ATM; and thus ATM regulates cell cycle and dictates whether cells survive or die with unrepaired DSBs [18]. 53BP1 and RNF168 also facilitates DDR. RNF168 is shown to ubiquitilate 53BP1 that is thought to have an overlapping role with XLF (DNA repair protein) [19]. Finally, DSBs are repaired by the DNA ligase IV (LIG IV)/XRCC4-XLF complex by NHEJ [20]. Recent research identified a paralog of XRCC4 and XLF (PAXX), and showed a redundant role of PAXX in DNA repair system (the entire process of V(D)J recombination is shown in Fig. 2).

B cells can undergo CSR, in which a set of IgH region is replaced by another Ig region, allowing B cells to produce different Ig subsets. CSR is initiated by activation of AID that deaminates cytidine to uracil at the transcriptionally active switch region [21]. Introduced uracil is modified by uracil-DNA glycosylase (UNG) and then removed by base excision repair. Abasic site is subsequently cleaved by apurinic/apyrimidinic endonuclease, generating DNA single strand break (SSB). Closely generated DNA SSB at the both strand lead to DSB. Alternatively, mismatch repair create DSBs by the contribution of MMR complex including MSH2, MSH6, MLH1, PMS2, and EXO1. The S μ /S α synapse is repaired with assistance of ATM/MRN/53BP1/RNF168/ γ H2AX, and then repaired with the same machinery used in the final process of V(D)J recombination [11]. Though hairpin is not formed in CSR process, Artemis is supposed to be involved by resolution of complex region generated in CSR [22, 23] (the entire scheme of CSR is shown in Fig. 2).

Disorders mainly present severe combined immunodeficiency (SCID) phenotype

RAG1/2 deficiency

RAG1 and RAG2 are absolutely required for V(D)J recombination; and thus mutations in *RAG1* or *RAG2* result is defect in B and T cells and show B-T-NK+ severe combined immunodeficiency (SCID) phenotype [6, 10]. Patients with partial loss of the recombination activity exhibits a variety of phenotype. These include Omenn syndrome (OS) that show erythrodermia, hepatosplenomegaly, lymphadenopathy, alopecia, and eosinophilia with elevated serum IgE. T cells are detected, but are usually oligoclonal. The reason for elevated IgE is largely unknown. Hypomorphic mutation in RAG1 is

Table 1 Immunodeficiency due to defects in DNA break induction, DNA damage response, non-homologous end joining, or other DNA repair systems

Function	Gene name	Alternative name	Disease name	Clinical phenotype	V(D)J, CSR	Immunological phenotype (human)
DNA break induction	RAG1/2		Severe combined immunodeficiency (SCID)	Can present Omenn syndrome when hypomorphic mutation	V(D)J	B-, T-, NK+
	AID		Hyper-IgM syndrome type 2	Lymphoproliferation	CSR	IgM↑, IgG↓, IgA↓, SHM↓
	UNG		Hyper-IgM syndrome type 5	Lymphoproliferation	CSR	IgM↑, IgG↓, IgA↓, SHM↓
	INO80		Hyper-IgM syndrome		CSR	IgM →, IgG↓, IgA↓, SHM↓
DDR/NHEJ signal	ATM		Ataxia telangiectasia (cellelular ataxia, telangiectasia, predisposition to malignancy)	Radiosensitivity, lymphoma, cerebellar ataxia, telangiectasia, endocrinological abnormality	V(D)J, CSR	IgA↓, IgE↓, IgG2↓, IgG4↓, low TRECs and KRECs
	ATR		Seckel syndrome	Microcephaly, short stature, cancer predisposition	(-)	(-)
	MRE11		Ataxia telangiectasia like disorder (ATLD)	Radiosensitivity, malignancy	?	No immunodeficiency
	RAD50		NBS like disorder	Radiosensitivity, malignancy, microcephaly	V(D)J, CSR	Panhypogammaglobulinemia, decreased T
NHEJ component	NBN	NBS1	Nijmegen-breakage syndrome (NBS)	Radiosensitivity, malignancy, microcephaly	V(D)J, CSR	Panhypogammaglobulinemia, decreased T
	53BP1		Not known in human	not known in human	CSR	Not known
	RNF168		Riddle syndrome	Radiosensitivity, dysmorphic features, short stature, mild motor impairment, intellectual disability	CSR	Panhypogammaglobulinemia
	PRKDC	DNAPKcs	Radiosensitive(RS)-SCID (close to DCLRE1C null)	Radiosensitivity, malignancy, neurological defect, autoimmunity	V(D)J, CSR	B-, T-, NK+
	KU70/80		Not known in human	Not known in human	V(D)J, CSR	Not known
	DCLRE1C	Artemis	RS-SCID	Mild radiosensitivity, no microcephaly	V(D)J, CSR	B-, T-, NK+
	LIGIV		DNA ligase IV syndrome	Radiosensitivity, malignancy, microcephaly, pancytopenia	V(D)J, CSR	B-, T-, NK+
	XRCC4			Microcephaly, facial dysmorphism	?	No immunodeficiency
	XLF	NHEJ1, Cernunnos	RS-SCID	Radiosensitivity, malignancy, microcephaly, developmental delay, pancytopenia	Mild defect	Lymphopenia, panhypogammaglobulinemia
	PAXX		not known in human	Not known in human	?	Not known
Others	FANC group		Fanconi Anemia	Pancytopenia, cancer predisposition	?	CVID phenotype (occasionally)
	MMR group		Constitutional Mismatch-repair deficiency syndrome (CMMRD)	Cancer predisposition	CSR	IgM↑, IgA↓, IgG↓

DDR DNA damage response, NHEJ non-homologous end joining

Table 2 Major manifestation of PIDs due to defective DNA repair system

Major manifestation	Gene name	Alternative name	Others
Reduced B and T (SCID)	RAG1/2		Can present OS
	PRKDC	DNAPKcs	
	DCLRE1C	Artemis	Can present OS, involved in CSR
	LIG IV		Involved in CSR
	XLF (mild)	NHEJ1, Cernunnos	
Class switch defect	AID		
	UNG		
	INO80		
	ATM		Involved in V(D)J recombination
	RNF168		Regulates 53BP1
	MMR group		
	FANC group		
Pancytopenia	XLF		HSC affected
	LIG IV		HSC affected
	ATM		
Malignancy	MRE11		Adenocarcinoma reported
	RAD50		
	NBN		
	RNF168		
	DCLRE1C		
	PRKDC		
	LIG IV		Can present OS
	XLF	NHEJ1, Cernunnos	
	FANC group		
	MMR group		
Microcephaly	MRE11		
	RAD50		
	NBN		
	LIG IV		
	XLF	NHEJ1, Cernunnos	

OS Omenn syndrome

reported in a patient with common variable immunodeficiency and in IgA deficiency [24, 25]. RAG1/2 deficient SCID is not radiosensitive, does not show any other developmental delay, but requires planning for hematopoietic cell transplantation (HCT) as soon as the diagnosis is made.

DCLRE1C (Artemis) deficiency

Artemis is phosphorylated by DNA-PKcs and by ATM, but is mainly activated by the former phosphatidylinositol (PI)-3-kinase. Defect in *DCLRE1C* gene is observed in B-T-NK+ radiosensitive SCID [26–29]. Mutations in *DCLRE1C* are usually observed in the N-terminal region leading to the loss of nuclease activity. The most frequent mutations (>50%) were large deletions of exons 1–3 or exons 1–4 due to a homologous recombination of

the wild-type *DCLRE1C* with a pseudo-*DCLRE1C* gene located 5' to the *DCLRE1C* start codon. Hypomorphic *DCLRE1C* mutation exhibits OS phenotype. Most of the patients with leaky SCID were compound heterozygous for one loss-of-function and one hypomorphic allele, with significant residual levels of recombination and DNA repair activity [29]. Patients with defective Artemis show predisposition to lymphoma. Radiosensitivity in Artemis deficiency is not prominent compared to that in LIG IV deficiency or XLF deficiency; and the patients do not show growth retardation or neurological deficits.

DNA ligase IV (LIG IV) deficiency

DNA ligase IV (LIG IV) is a ligase that resolves the DSBs during V(D)J recombination among three DNA ligase family. LIG IV deficiency was originally found in

Fig. 1 DNA repair proteins involved in V(D)J recombination. RSS recombination signal sequence

a patient with leukemia who showed severe side effect of chemotherapy. Subsequently, the disorder was reported in patients with microcephaly, growth retardation, pancytopenia, and various degree of immune dysfunction. Commonly associated features include growth failure, severe microcephaly, pancytopenia and a predisposition to lymphoid malignancy [30]. Most cases reported harbor missense *LIG IV* mutations or nucleotide deletions displaying a variety of presentation. The missense mutations with reduced ligase IV activity (typically 5–10%) present combined immunodeficiency. The patients show a wide range of immunological abnormality with decreased B, T, and NK cells.

XLF (NHEJ1, Cernunnos) deficiency

XLF deficiency was identified in patients with radiosensitive SCID and cytopenia [31]. It was postulated that XLF is critical in V(D)J recombination given the fact that it is a component of LIG IV complex; however, the immune

system is relatively mildly affected compared to other SCID. Recent study demonstrates that XLF is dispensable for V(D)J recombination [32]. After cleavage in vitro, the RAG proteins remain associated with the DNA ends in a post-cleavage complex (PCC), which serves a crucial function in joining coding and signal ends. The RAG complex may play a role in assisting repair of RAG-mediated DSBs in the absence of XLF [32]. XLF, instead, stimulate N-nucleotide insertion hereby generating receptor diversity [33]. Defective XLF is associated with impaired survival and premature aging of hematopoietic stem cells, leading to cytopenia [34, 35].

XLF-related molecules and phenotype of the defect (non-SCID)

XRCC4 and XLF are a member of the same protein family and show structural similarity playing a role in NHEJ in concert with LIG IV. It has been postulated that defect in XRCC4 would display a phenotype of SCID as observed

Fig. 2 DNA repair proteins involved in Immunoglobulin class switch recombination (CSR). *AID* activation induced cytosine deaminase, *APE* apurinic/apyrimidic endonuclease, *UNG* uracil *N*-glycosylase

in immunodeficiency caused by a defect in XLF or in LIG IV. Recent study has identified a compound heterozygous mutation in *XRCC4* in a patient with microcephaly and cerebellar ataxia with normal immune cell composition and response [36]. The cells showed severe defect in DSB repair, but displayed normal V(D)J recombination capacity using an artificial substrate.

PRKDC (DNA-PKcs) deficiency

Gene analysis of B-T-NK + SCID patient without any developmental delay or mutation in *RAG1/2* revealed homozygous mutation in *PRKDC* in 2009 [27]. The mutated DNA-PKcs did not show normal protein expression and possessed normal kinase activity, but lacked in a capacity to activate Artemis. Another *PRKDC* deficient patient showed reduced DNA-PKcs protein expression, undetectable kinase activity, and impaired DSB rejoining. The patient also exhibited microcephaly, developmental delay, and severe neurological deficit [37]. These findings indicate DNA-PK is critical in neurological development. Mutated DNA-PKcs failed to induce autoimmune regulator (AIRE)-dependent gene expression in vitro, which

correlated with the presence of anti-calcium sensor receptor antibody in the patient. DNA-PKcs and KU70/80 are particularly abundant in human; and may play an additional role in genome stability such as protection of telomere damage. In fact, mice or dogs lacking in DNA-PKcs display SCID phenotype but show almost normal growth and neurological development.

Disorder mainly present CSR deficiency

AID deficiency and UNG deficiency

The most common identified cause of autosomal recessive hyper-IgM syndrome results from defects in the *AICDA* gene encoding for AID, an enzyme required for CSR and somatic hypermutation (SHM) in B cells [7, 38, 39]. Expression of AID is upregulated in response to signal through CD40 in B cells and it initiates CSR by deaminating deoxycytosine in the switch regions of the IgH genes generating deoxyuracils in both DNA strands. UNG then removes deoxyuracils from DNA and initiates the DNA repair pathway. Defects in UNG also result in hyper-IgM

syndrome [7, 38]. AID deficiency and UNG deficiency have lymph node hyperplasia caused by the presence of giant germinal centers. Six of 22 patients with AID defects had autoimmunity or inflammation: diabetes mellitus, polyarthritis, autoimmune hepatitis, hemolytic anemia, immune thrombocytopenia, Crohn's disease and chronic uveitis.

INO80 deficiency

Defect in INO80 was recently reported in two patients with normal IgM, decreased IgG and IgA [11]. INO80 is a component of chromatin remodeling complex and is involved in DNA repair. The presence of both INO80 and cohesion subunit SMC were detected on S α and E μ regions in model B cells, suggesting the role of INO80 in S-region synapsis during CSR. INO80 may modulate cohesin activity in B cells.

Mismatch repair deficiency syndrome

Constitutional mismatch repair deficiency (CMMRD) syndrome is a disorder associated with a high risk of malignancy [40]. Causative mutations are detected in DNA MMR genes, *PMS2*, *MSH6*, *MSH2* or *MLH1*. The mutations are reported in patients with hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome. CMMRD is characterized by childhood brain tumors, hematological malignancies, and gastrointestinal cancer later in life. Modest decrease of IgG, particularly IgG2 subclass, but not IgA was reported in *MSH6* deficiency [41]. The defect leads to a partial Ig-CSR defect and an abnormal SHM pattern. *MSH6* seems to be not only able to convert staggered DSBs to blunt DSBs but is also involved in S junction DSB repair.

RIDDLE syndrome (RNF168)

RIDDLE (radiosensitivity, immunodeficiency, dysmorphic features, and learning difficulties) syndrome is a novel immunodeficiency disorder associated with defective DSB repair [42, 43]. Immunologically the patients showed decreased IgG and IgA. RIDDLE syndrome shares overlapping clinical features with ataxia telangiectasia (A-T). The cells from a RIDDLE patient exhibit impaired relocalization of 53BP1 and BRCA1 to DSBs, while MDC1 and NBS1 remain unaffected. The responsible gene for the syndrome is RNF168 that mediates ubiquitylation of 53BP1 [19]. The modification is required for the recruitment of 53BP1 at the sites of DSBs and for its function in DNA damage repair, checkpoint regulation and genomic integrity.

Disorders involved in DDR and NHEJ signaling

Ataxia telangiectasia

Ataxia telangiectasia (A-T) is an autosomal recessive neurodegenerative disorder characterized by early onset ataxia, telangiectasia at around 6–7 years old, and an increased incidence of malignancy, and the disorder is caused by mutation in *ATM* [44, 45]. About 30% of A-T patients exhibit immunodeficiency, showing decreased T cell number due to impaired efficiency in V(D)J rearrangement and decreased T cell neogenesis. Decreased serum IgG2 and IgA are also noted, which is caused by impaired CSR. Increase of serum alpha-fetoprotein (AFP) and chromosomal translocations involving chromosomes 7 and 14 are frequently noted in A-T patients. The most prominent feature of A-T is radiosensitivity. *ATM* kinase plays a central role in DDR by phosphorylation of various DDR molecules, and is involved not only in DSB repair, cell cycle regulation, cell fate decision, but also in transcriptional regulation, telomere maintenance, mitochondria maintenance, nonsense mediated decay, and autophagy [2, 46]. Defect in *ATM* leads to predisposition to malignancy, especially to leukemia and lymphoma.

M/R/N complex deficiency

Deficiency in NBN(NBS1) is known as Nijmegen Breakage syndrome (NBS), a chromosomal instability syndrome characterized by microcephaly, “bird-like” face, growth retardation, mild mental retardation, immunodeficiency, and a predisposition to malignancy [47]. Immunological studies reveal mild or severe leukopenia in nearly half of the patients with evident reduction in lymphocytes. Absolute number of T cells decrease in majority of patients, accompanied by a reduced CD4+ T cells. The number of B cells is reduced in three quarter of NBS patients. The humoral immunodeficiency in NBS patients ranges from agammaglobulinemia to a moderate reduction in the immune response. IgG2 subclass deficiency is also reported in NBS, indicating involvement of NBN in CSR.

Defect in *RAD50* resembles phenotype of NGS. The patients presented microcephaly, mental retardation, ‘bird-like’ face, and short stature, but had normal lymphocyte counts and immunoglobulin level [48].

Patients with *MRE11* deficiency display Ataxia telangiectasia like disorder (ATLD) with ataxia, cerebellar atrophy, ocular apraxia, but without telangiectasia [49, 50]. No immunological abnormality has been reported so far; but the disorder show predisposition to malignancy.

MRE11, RAD50, and NBN function as a M/R/N complex at DDR signal; and each component is indispensable for the stable complex formation. However, contribution of each component to immune response, especially in V(D)J recombination and CSR could differ.

Other genomic instability disorders that can exhibit immunodeficiency

Fanconi anemia (FA) is a heterogeneous disorder that produces a range of phenotypes that are highly variable. These include bone marrow failure (pancytopenia), developmental delay, malformation, and high prevalence of hematological malignancies [51]. At least 19 genes are found to cause FA so far [52]. The protein encoded by the genes function together in a common pathway called as the FA pathway. The FA pathway uses components of other known DNA repair processes to achieve proper repair of ICLs [53]. In addition, Fanconi anaemia proteins have functions in genome maintenance including the stabilization of replication forks and the regulation of cytokinesis. Recent study (Sekinaka Y, et al. *J Clin Immunol*, in press) has demonstrated that FANC mutations are involved in impaired lymphogenesis probably by the accumulation of DNA replication stress, leading to phenotype of common variable immunodeficiency. Some of the patients show undetectable T-cell receptor excision circles and Kappa deleting recombination excision circles.

Therapeutic measures for immunodeficiency due to defective DDR

Patients with B-T-radiosensitive SCID phenotype regardless of presence or absence of NK cells suffer from recurrent or severe infection and thus require reconstitution of their immune system as a curative measure. These disorders include deficiency in DCLRE1C (Artemis), DNA ligase IV (LIG IV), XLF (Cernunnos). Results of hematopoietic cell transplantation for radiation-sensitive SCID suggest that minimizing exposure to alkylating agents and ionizing radiation is important for optimizing survival and minimizing late effects [54]. LIG IV deficiency and XLF deficiency hardly survive myeloablative conditioning regimen, thus needs reduced intensity conditioning regimen. On the other hand, those with residual NK activity can reject donor hematopoietic cells. Artemis deficiency does not have hematopoietic stem cell defect, necessitating agents to remove host hematopoietic cells and to open marrow niches before the transplantation. One of the conditioning regimen currently used is a combination of Flurabine, low dose cyclophosphamide, and serotherapy to remove T

cells. Development of non-genotoxic conditioning regimen to suppress residual NK cell activity and generate marrow niches is awaited.

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