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RAG1: THE CORNERSTONE GENE INVOLVED IN VARIOUS IMMUNE DISEASES AND CANCERS

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RAG1 is an abbreviation for recombination activating gene-1 which is the cornerstone in the process of V(D)J recombination. The process enables our immune system to produce an unlimited number of antibodies and T-cell receptors to fight almost any infection. RAG1 mutations are greatly involved in the development of primary immunodeficiencies (PID) including the early-onset severe combined immunodeficiency and Omenn Syndrome. Additionally, RAG mutations are found in delayed-onset cases of combined immunodeficiency with autoimmunity and/or granulomas. The early-onset category occurs due to severe immunodeficiency and the delayed-onset one occurs due to immune dysregulation. Furthermore, RAG1 abnormalities are found in several hematological neoplasms such as acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS) and lymphoma. The mechanism by which RAG1 contributes to different types of cancer is not fully understood. In this review, we display the role of RAG1 in the different diseases digging to understand how it works and whether it can be a promising biomarker for the progression of some of these conditions.

Keywords: *RAG1; V(D)J recombination; Mutations; Immunodeficiencies; Leukemia*

INTRODUCTION

Despite the innate immunity strength in protecting our body, the extent of pathogenic elements that can be caught by innate immunity is limited. The evolution of adaptive immune system has been driven by the huge variability of pathogens and the ability of pathogen to greatly mutate to bypass the host immune system[1]. The main difference between innate and adaptive immunity is that all innate immune system recognition receptors are encoded in their mature form in the germline genome, while the adaptive immunity relies on recognition receptors largely changeable according to a dynamic process of somatic recombination of large range of gene segments. After the initial exposure to a pathogen, cells expressing specific receptors against the pathogen can persist in the host for lifetime, giving memorial and robust defense in case of re-exposure[2].

The effector cells of the cellular immune responses of the adaptive immune system are called lymphocytes. They are developed in the primary lymphoid organs including thymus for T-lymphocytes and bone marrow for B-lymphocytes, then they move to the secondary lymphoid organs as lymph nodes and spleen which oblige to catch pathogens from lymph and blood, respectively. These areas are the starting point of the adaptive immunity under the effect of innate immunity signals exerted directly by the passing pathogens or indirectly by the antigen presenting cells (APCs) trafficking to the secondary lymphoid organs. T and B Lymphocytes can leave the secondary lymphoid organs to other parts of the body to employ effector functions under regulation on various adhesion molecules and chemokine receptors[2].

T cells develop from common progenitor cells migrating from bone marrow or fetal liver to the thymus[3-5]. Once arrived, the progenitors rapidly expand under the effect of interleukin-

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7(IL-7). IL7 is thought to regulate expression of our gene of interest (RAG1). This expansion is followed by launch of transcription factors mainly notch-1 which is originally a transmembrane protein upon activation stimulates development of the T-cell lineage and expression of genes essential for T-cell receptor (TCR) assembly, one recent study stated that RAG1/2 might be a target for Notch1. After expansion of T-cell progenitors or pro-T cells in the thymus, differentiation occurs through an antigen-independent process of genomic rearrangement to create the efficient genes encoding the various chains ((α and β) or (γ and δ)) of TCR. Ninety percent of cells have α and β chains.

Each of α and δ chains contains V (variable) and J (joining) segments in addition to C (constant) segment. While β and γ chains are comprised of V, D (Diversity), J and C segments. Huge numbers of TCR are obtained through the V(D)J recombination process which will be discussed later in detail. The transition from (double negative (DN)) pre-T cell to a double positive (DP) T cell is marked by the surface expression of an α β and γ δ TCR resulting from the sequential productive rearrangement of 2 TCR genes. Double positive cells express both clusters of differentiation 4 (CD4) and CD8. The TCR chains form a complex at the cell surface with the protein creating CD3, including the γ, δ, ε and ζ chains. TCR structure is shown in **Fig. 1.** Moving to B cells, they originate from hemopoietic stem cells (HSC) in the bone marrow. Develop of the B-cell lineage is under the control of several transcription factors such as IKAROS (a DNA-binding protein encoded by IKZF1and a member of a family of restricted zinc-finger transcription factors)[6&7]. The appearance of both IgD and IgM marks the mature antigen naïve B-cells. The genes of immunoglobulins are assembled in a way like TCR genes through V(D)J recombination process. Heavy chains (e.g., IgM, IgD, IgG1-4, IgA1 and IgA2, and IgE) are composed of 4 segments (V_H, D, J_H, and C_H). While light chains (e.g., κ and λ) have 3 segments (V_L, J_L, and C_L). Immunoglobulin structure is shown in **Fig. 2.**

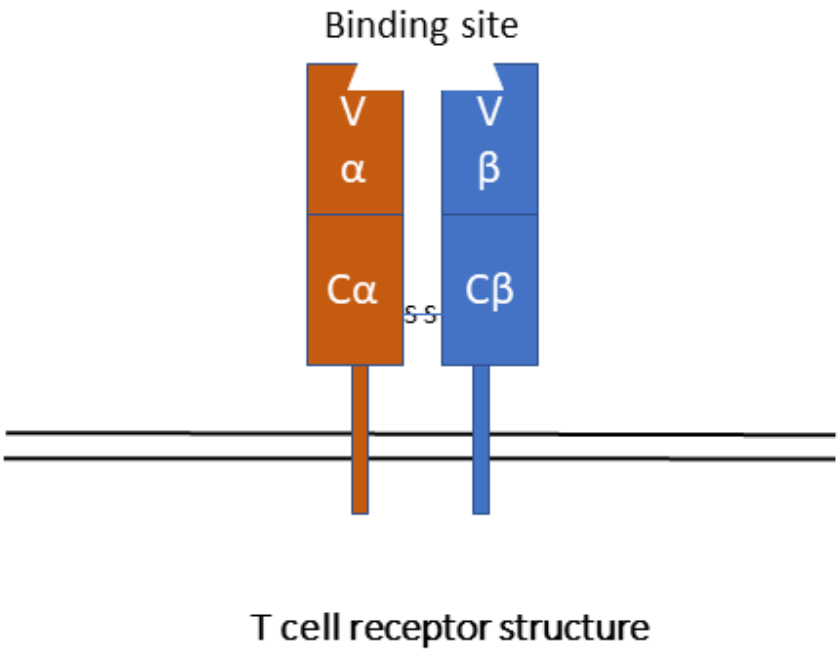


Fig. 1: T-cell receptor structure. It consists of two bisulfite-linked polypeptide chains, α and β (corresponding to light and heavy chains of an antibody, respectively) or δ and γ and it contains one binding site. They are always membrane-bound, each chain contains one variable domain, one constant domain followed by transmembrane domain and a short intracellular tail.

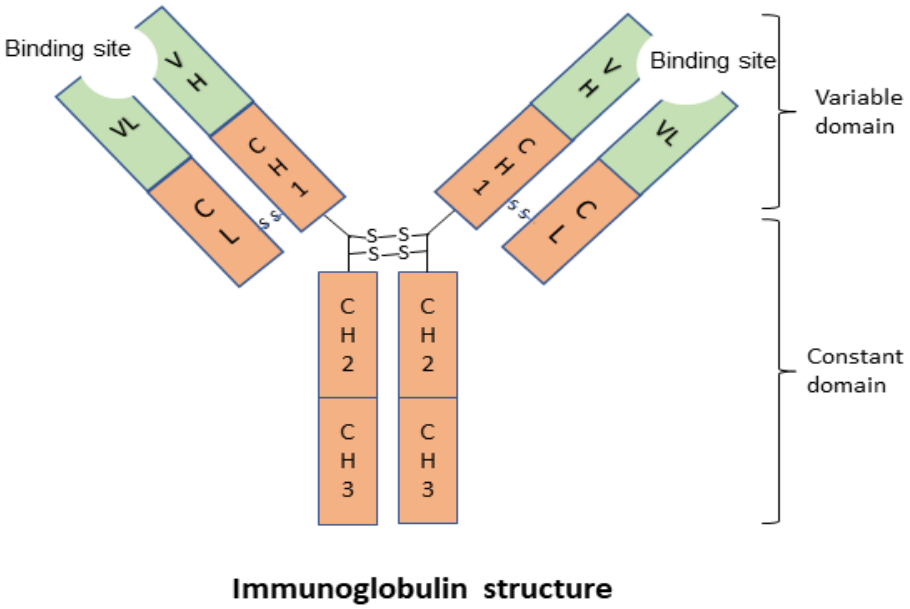


Fig. 2: Immunoglobulin structure. Antibodies are comprised of four polypeptide chains, two heavy and two light, connected by disulfide bonds. There are two parts, the variable domain consisting of the antigen binding site, and the constant region represents the effector function of the antibody.

The number of unique antibodies and TCRs that can be found within the human body at any given time is estimated to be upwards of one billion[8]. Specifically, the human body can possibly produce more than 10^{12} distinct antibody molecules even in the absence of antigen stimulation, which is called preimmune antibody repertoire[9]. Given that the human genome contains fewer than 50.000 genes, what is the cause behind the production of such an immense array of antibodies? The answer lies in the process of mixing and matching the different segments of genes encoding each chain. During B and T cells development, the V and J gene segments (for the light chain in case of B cells and for α and δ chains in case of T cells) and the V, D and J gene segments (for the heavy chain in case of B cells and for β and γ chains in case of T cells) are joined together to form a functional V_L- , V_H-region or TCR coding sequence by a process called V(D)J recombination. This process is partly responsible for production of a huge repertoire of antibodies. In B cells, there are other two processes that contribute to the Ab diversity occurs after the migration of B cells to the germinal center of the lymph node and after their binding to an antigen. One of them is the process of somatic hypermutation (SHM) which induces highly localized point mutation by the activity of AID (activation induced cytidine deaminase) to increase the precision of the Ab-Ag interaction. SHM does not take place in the T cell, so V(D)J recombination process is entirely responsible for the TCRs diversity, and it also explains the relative low affinity of TCR-Ag interactions compared to Ab-Ag interactions. Lastly, the process of class switching aims to change the type of an Ab (from IgM or IgD to IgE or IgG) by inducing changes in switch (S) region located upstream the region encoding the heavy chain of the constant region (C_H) under the regulation of AID[10].

Our gene of interest (RAG1) works collaboratively with another gene called RAG2 which regulates RAG1 binding to the active site during the recombination process. Mutations in both genes were frequently reported in patients with primary immunodeficiencies. However, RAG mutations were not reported in hematological disease, changes in RAG1 expression were reported in hematological cancers like acute lymphocytic

leukemia (ALL) and the deterioration of chronic myeloid leukemia (CML) and myelodysplastic syndrome. In this review, we show the significant role of RAG1 in maintenance of our immune system and how its role is expanded to include other diseases.

The process of V(D)J recombination

To defend almost any pathogen, our immune system has the ability for production of large range of antibodies (Ig) and T-cell receptor (TCR) genes. The reason for this unique ability is a process called V(D)J recombination occurring for V(Variable), D(Diversity) and J (Joining) gene segments in case of Ig and TCR heavy chain or between V and J in their light chain.

These segments are neighbored by one, or two in case of D segment, conserved DNA sequence called recombination signal sequence (RSS). RSS consists of three elements; two well conserved domains; heptamer (5`-CACAGTG-3`) and AT-rich nonamer, separated by a less conserved spacer whose length is either 12bp or 23bp, accordingly, RSS is referred to as 12-RSS or 23-RSS, respectively[11]. V(D)J recombination preferably occurs between a 12-RSS and a 23-RSS, this is known as the 12/23 rule[12].

V(D)J recombination starts with the binding of RAG1/RAG2 hetero tetrameric complex with one RSS. Then the RAG-RSS complex paired with a second RSS in a process known as synapsis. Within this synaptic complex is paired, DNA cleavage starts on one strand and in the presence of nucleophile as a nick is introduced at the 5` of the heptamer leaving 3` hydroxyl group on the coding segment and 5` phosphate on the RSS. Then the 3`-hydroxyl group attacks the other strand by transesterification reaction forming phosphodiester bond producing a DNA hairpin intermediates[13&14]. This process creates two types of ends; hairpin coding ends of the coding gene segments (V, D or J) and blunt signal ends of RSS terminating with the heptamer**.** The nicking step may happen before or after synapsis, but the double strand breaks happen exclusively within the synaptic complex[15].

Coding ends and signal ends (the latter is mostly held with RAG protein in a post-cleavage complex (PCC)) are exposed to the non-homologous end joining mechanism (NHEJ) to finally generate coding joint and signal joint, respectively[16&17]. Recruitment of the three members of DNA-dependent protein kinase (DNAPK); Ku70, Ku80 and the catalytic subunit DNAPKcs along with XRCC4-DNA Ligase IV complex and Artmis protein is necessary for the NHEJ pathway. Due to the different nature of ends and different configuration, coding ends and signal ends were treated with slightly different mechanisms. The coding ends are firstly bound with the previously formed heterodimer (Ku70:Ku80) which stimulates the recruitment of DNAPKcs: Artemis complex[18]. The endonuclease activity of the latter opens the hairpin randomly at any point away from the apex so the strand extends with some or all of the nucleotide of the complementary strand according to the site of the cut, creating palindromic (P) nucleotide overhang[19]. Other source of diversity is created by a lymphoid specific polymerase called terminal deoxynucleotidyl transferase (TdT) which adds template-independent deoxy nucleotides known as non-templated (N) nucleotides[20]. After removal of the non-complementary nucleotides by exonuclease and filling any gaps by DNA polymerase (DNA Pol μ or DNA Pol λ), the two ends were ligated by DNA ligase IV: XRCC4 to form the coding joint. Due to their blunt ends, the signal ends were processed in a simpler way. Firstly, the Ku70:Ku80 dimer binds to the signal ends, then DNA ligase IV: XRCC4 complex is recruited and facilitates the joining of the two ends to form the signal joint. According to the orientation of the RSSs, the signal joint is integrated in the genome or removed as an excised signal circle (ESC). If the two RSSs are oriented in opposite directions (convergent RSSs), deletional recombination happens and the ESC is formed as in **Fig. 3A**. If the two RSS are oriented in the same direction (co-oriented), inversional recombination happens and the signal joint is retained in the genome **Fig. 3B**[21].

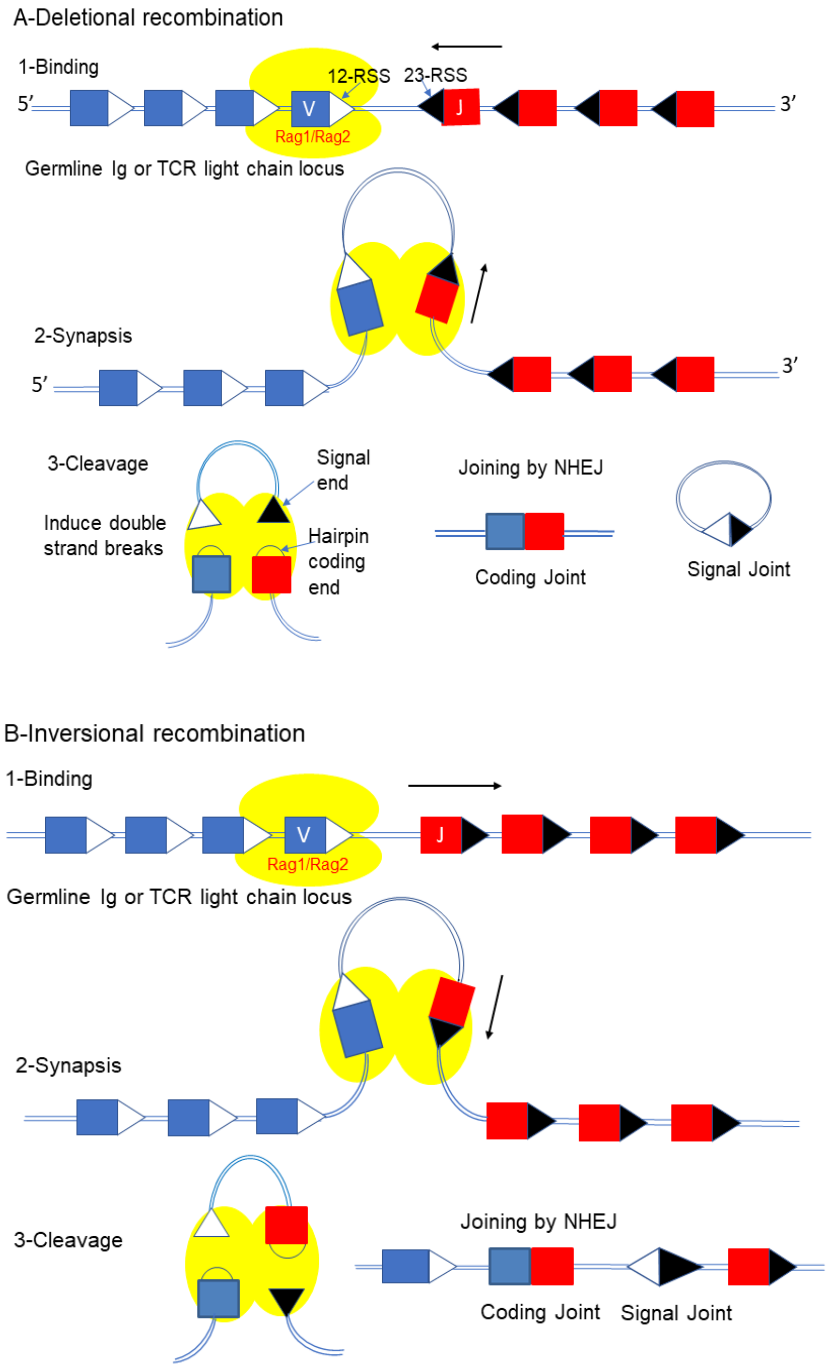


Fig. 3: A schematic presentation for major steps of V(D)J recombination. The process starts with binding of RAG1/RAG2 complex with one of the RSS, then synapsis through incorporating the second RSS. Next, cleavage happens between the RSS and the coding segment leaving blunt signal ends and hairpin coding ends which finally are processed by NHJH to give either excised signal joint in case of convergently directed RSS as in the deletional recombination(A) or integrated signal joint into the genome in the case of co-oriented RSSs as in the inversional recombination (B).

(V segments are represented by blue rectangles, J segments are red rectangles, 12-Rss are white triangles and 23-RSS are black triangles).

RAG structural domains

The human RAG1 and RAG2 genes are located on chromosome 11p13. Both genes contain only one protein-coding exon. During T cell and B cell development, many cis-enhancer elements upstream of RAG2 activate the transcription of RAG1 and RAG2 in co-ordination[22].

Both RAG proteins have an enzymatically active core portion[23] in addition to the non - core portion. Different structural and functional domains have been identified along the core and the non-core regions. The major domains of RAG proteins are well known especially after the availability of its high-resolution structure. RAG1 protein consists of 1040 aminoacids which can be truncated to core RAG1 (cRAG1) extended from aa 384 to 1008. Most studies have used cRAG1 instead of the full length RAG1 (flRAG1) because cRAG1 is sufficient for V(D)J recombination and it is much easier to purify[24].

Nevertheless, insertion of cRAG1 in mice instead of wild type RAG1, a procedure known as knock-in, resulted in minor impairment in the V(D)J recombination and the lymphocyte maturation[25] referring to the importance of the non-core region. The structure of cRAG1 is thoroughly understood. The core region contains seven main modules; the nonamer-binding domain (NBD) interacts with the RSS nonamer, the dimerization and DNA binding domain (DDBD) connects to NBD via a flexible linker and helps together in RSS binding and in RAG1 dimerization to form the RAG complex, the other five domains (PreR; Pre-RNase H, RNH; RNase H; ZnC2, ZnH2 and CTD; carboxy-terminal domain) represent the active catalytic site containing three conserved DDE motif, acidic residues (D600, D708 and E962)[26] that coordinate the divalent metal factor, most likely magnesium ions in the RAG1 active site[27-29]. D600 and D708 are in RNH while the last residue, E962 is located after two important zinc binding modules, ZnC2 and ZnH2. These two modules contain pair of cytosine (C727 and C730) and pair of histidine (H937 and H982) residues which together form Zn binding site which is important in the dimerization of RAG1[30] and essential for DNA cleavage[31]. The final module, the CTD, folds back to collaborate with the DDBD in the DNA binding and RAG1 dimerization[26,32&33].

Most of non-core region is in the N-terminus of RAG1 protein. Compared to cRAG1, the structure of non-core RAG1 is less understood. However, the domains of this region are well recognized. A region of basic aminoacids in the N-terminus of the non-core region has been reported to involve in the process of Zinc binding and to contact with the nuclear import proteins SRP1 and Karyopherin alpha 1 (KPNA1; also known as importin subunit α5) which facilitate nuclear localization of RAG1[34&35] But, it is not the only way for the nuclear import[35] as the core provides a nuclear localization signal to interact with KPNA1[36].

Another important domain is known as C3HC4 RING (really interesting new gene) finger domain with H3 ubiquitin ligase activity[37]. RING domain shows the important regulatory role of the non-core as the RAG1 ubiquitin activity is closely related to the overall V(D)J recombination[35,38]. Mutation of a key cysteine residue (C325) results in loss of RAG1-mediated ubiquitin ligase activity and shutdown the autoubiquitination of lysine residue (K233) in RAG1[39] and the RAG-mediated ubiquitylation of Histone H3[40&41]. The interaction of RAG1 with unubiquitylated H3 has been found to limit RAG1 binding activity and H3 ubiquitylation would liberate RAG1 and restore catalytic function[42].

RAG2 is known to have important regulatory role to the V(D)J recombination by guiding and controlling the RAG1 binding and cleavage activity by increasing its specificity to RSSs[43]. Like RAG1, the full length RAG2, 527 aminoacids, can be truncated to cRAG2, extending from the N- terminus to aa 382, and is made up of a six-bladed β-propeller like structure (antiparallel β-sheets). Each blade formed by a Kelch-like motif[44]. The flat surface of the "doughnut" shaped core is appropriate for mediating protein-protein interaction[45]. Although RAG2 does not bind the DNA directly in the absence of RAG1, RAG2 binds with RAG1 near its active site, stabilizes the interaction between RAG1 and RSS heptamer and controls DNA cleavage[46].

The non-core RAG2, like non-core RAG1, has a regulatory effect. It extends from aa 383 to the C-terminus. The most prominent domain

in the non-core region is a plant homedomain (PHD) finger located (from aa 414 to 487), separated from the core region with acidic hinge and followed by a C-terminal extension[44]. The RAG2 PHD finger binds to trimethylated lysine 4 on histone H3 which marks the points of active chromatin and guides the RAG complex towards the accessible RSSs[47], leads eventually to enhancement of the binding and the cleavage activity of RAG1-RAG2 complex[48-50].

The acidic hinge offers allosteric inhibition for RAG1 binding with RSS. Binding of PHD to H3K4me3 induces some conformational changes in both RAG1 and RAG2 and counteracts the autoinhibition effect of the acidic hinge[50]. The final part of non-core RAG2 is the C-terminal extension and its main function is the regulation of RAG2 degradation in the cell cycle[51]. RAG1 and 2 structures are shown in **Fig. 4A and B**.

Better understanding for the phenotypic diversity has been recently achieved through the new advanced methods to assess the expression and function of mutated RAG proteins. The heterotetrametric complex structure of RAG1/2 core domains have been recently solved by using the crystallographic[52] and cryo-electron microscopy[53]. Both molecules of RAG1/2 form a Y-shaped structure whose stem is the NBDs of the two RAG1 molecules, and the DDBDs form the branch point. Therefore, the two RAG1 molecules spread out, the zinc-binding region projects upwards[28&29]. The six-bladed β-propeller of core RAG2 binds directly with the RAG1 domains beyond the DDBD including the CTD[52], forming the two branches of the Y-shaped structure of the RAG complex (**Fig. 5**). After DNA binding, the two parts of RAG complex become nearer[53]. Although RAG2 does not bind directly to the RSS nonamer, it contributes to the stability of RAG complex/RSS heptamer binding[53].

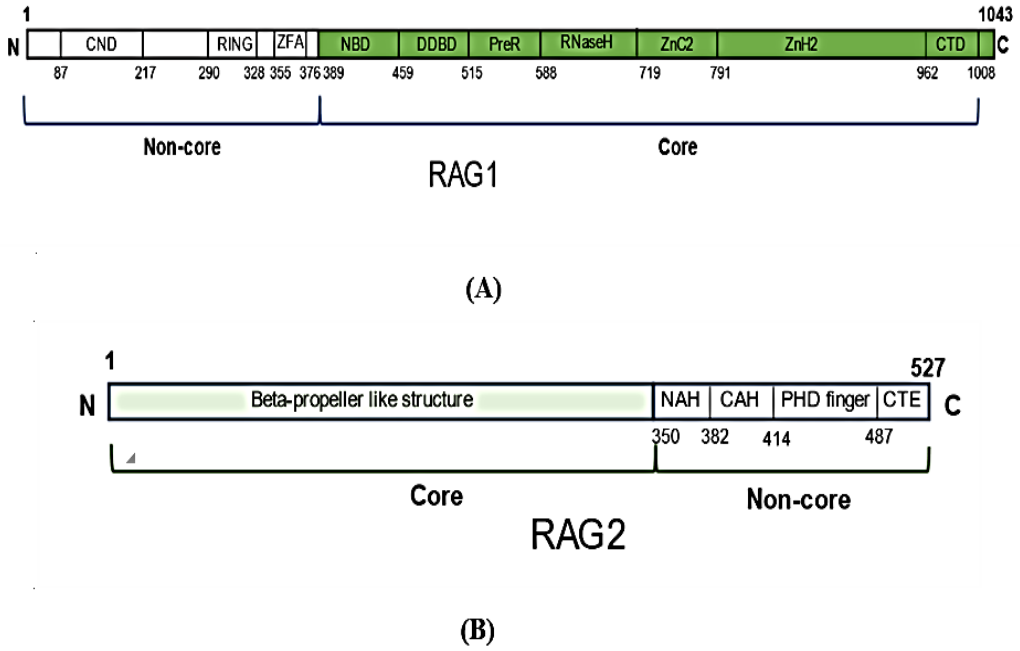
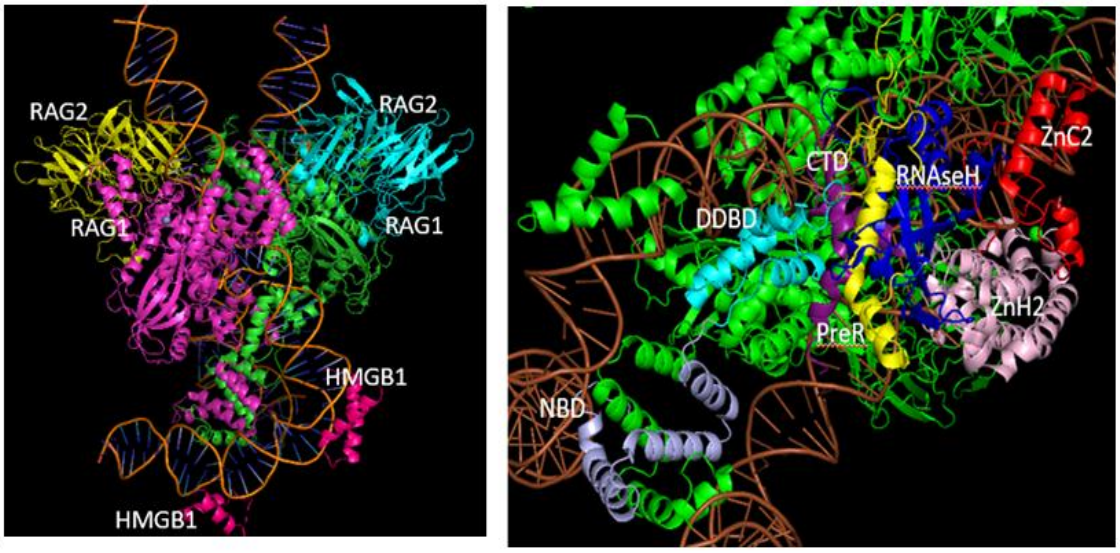


Fig. 4: Structure of RAG1 and RAG2. Functionally, Each RAG protein has two parts: core (green) and noncore domains (white).

(A-RAG1 noncore domains; CND, central non-core domain; RING, really interesting new gene, ZFA, zinc finger A, Core domains; NBD, nonamer binding domain; DDBD, dimerization and DNA binding domain; PreR, Pre-RNAse H; CTD, C-terminal domain)

(B-RAG2 Core domain, non-core domains; NAH; N-terminal acidic hinge, CAH, C-terminal acidic hinge, CTE; C-terminal extension).



(A) **(B)**

Fig. 5: (A) Different chains in the cryo-structure RAG1/2 in complex with nicked DNAs (protein data bank identification code (PDB)=6cg0) along with 2 molecules of high mobility group box 1 (HMGB1)[54] displayed on Pymol program**.** (B) Later side view for the different domains of RAG1 each domain is differentiated by color using Pymol program.

Consequences of RAG deficiency/ abnormalities

Many clinical phenotypes are associated with RAG mutations and accompanied with infections and autoimmunity. Additionally, environmental factors have a chief role in the diversity of these phenotypes.

T cell negative B cell negative SCID and Omenn syndrome

SCID is an abbreviation of severe combined immunodeficiency which includes many distinct disorders caused by severe defects in the development and function of T cells which may extend to affect B cells in some cases[55]. T cells are lower than 300 cells/ml with absent B cells. This condition is certainly life-threatening early in life, unless immune reconstitution is accomplished, usually with HSCT (HSC Transplantation)[56]. The T−B− SCID with normal cellular radiosensitivity was discovered to be caused mainly by RAG mutations[57]; While T −B− SCID with increased cellular radiosensitivity was believed to be associated with mutations in genes encoding various elements of the NHEJ pathway[58].

Regarding Omenn syndrome(OS), It was described in 1965 for the first time[59]. Patients with Omenn syndrome have symptoms of early-onset generalized erythroderma, lymphadenopathy, eosinophilia, hepatosplenomegaly and severe hypogammaglobulinemia with elevated IgE levels, which are accompanying with the presence of autologous, oligoclonal and activated T cells that permeate many organs with absent B cells[60&61].The existence of both OS and SCID (with T^- and B^-) in two siblings pushed the search for RAG mutations in patients with OS[60]. Hypomorphic RAG mutations that cause reduction but not complete suppression for the recombination activity were proved to be the most frequent cause of OS in humans[62]. If there are suboptimal autologous T cells with clinical symptoms of OS in patients having hypomorphic RAG mutation[57,63], this condition is referred to as (atypical or leaky) SCID[58,64].

Another SCID phenotype including the oligoclonal expansion of autologous γδ T cells (known as γδ T+ SCID as T cells express γδ TCR) was then described in infants with RAG deficiency and infected with cytomegalovirus (CMV)[65&66]. Most of these patients have measurable B cells, autoimmune cytopaenias and are at high risk for Epstein–Barr virus-induced lymphoproliferation. Patients with SCID, AS and OS have high risk to all types of life-threatening infections, and they may

experience severe complications after administration of live vaccines. In a screening for the SCID in the United States newborns, one from each 58 hundred experienced SCID and associated diseases in which RAG mutations represent 19% and they are the main cause of atypical SCID (AS) and Omenn syndrome[67]. This frequency increases in countries with elevated rate of parental consanguinity[68].

The diminished immune competence associated with RAG mutations confirms the vital role of RAG and V(D)J recombination in the development of T and B cells and the diversity of their repertoire. However, the detection of various phenotypes for the same RAG mutation[63, 69,70] has demonstrated the contribution of other factors in the disease phenotype. These factors include the environmental factors for example, γδ T cells were reported to be expanded in RAG-mutated infants with CMV infection, in addition, parainfluenza type 3 virus infection contributed in the conversion of SCID to OS in a RAG-deficient infant[71].

Other milder/delayed onset types

Moving to milder and more delayed onset diseases than SCID, OS and AS. Three girls were reported in 2008 to have granulomas in the skin, mucous membranes, and internal organs. Additionally, they experienced several viral infections and developed B-lymphoma. They had low count of B and T cells, but they maintained diverse TCR repertoire, and they tolerated vaccination. Other cases with RAG mutations and delayed-onset manifestations were reported and this disorder has been known as CID–G/AI (combined immunodeficiency with granulomas or autoimmunity)[72]. The experienced autoimmunity is due to the defective T and B cells tolerance, the process in which the autoreactive T-cells and the autoantibodies are removed. This type of disorder has been described under the term 'Immune dysregulation'[73]. In vitro recombination studies correlated level of RAG activity and the clinical phenotype[74].

How to solve RAG deficiencies?

Supportive treatment (antibiotics, Ig substitution therapy, etc) are needed for patients with severe forms of RAG deficiency (SCID, OS and atypical SCID), however HSCT is the main therapeutic approach till now. The best outcome has been achieved when the donors are matched related, lower outcomes has been observed with haploidentical donors[75]. These results has been encouraging for development of Gene therapy which has shown good results in treatment of X-linked SCID[76&77] and deficiency of adenosine deaminase[78&79]. T cell lineage has a relative benefit for gene-corrected cells in both conditions. Trials of gene therapy in RAG1 deficient mice have shown controversial results with severe complications like development of acute lymphoblastic leukemia[80-82]. There is an emerging need for development of novel vectors for development of RAG1 Gene-therapy strategies. Better experimental results have been observed in RAG2- gene therapy[83&84]. These results give courage to the development of gene therapy for RAG2-deficient individuals soon, but further research is needed to exclude the presence of competition, in case of hypomorphic RAG2 mutations, between the self, mutated lymphoid progenitors and the gene -corrected cells which may hinder the success of the immune reconstitution.

Malignancies with dysregulated RAG expression

RAG's role in development of malignancies was proven in different previous studies. Off-target RAG binding is one of the illustrations as RAG1 non-specifically binds to RSS- similar sequence called 'cryptic RSS' which spread all over the genome and induces DNA Double strand breaks (DSB) which ultimately leads to chromosomal instability[85]. Kirkham, C.M et al suggested a mechanism called (cut-and-run) as a cause for increase DSB by RAG and the process of V(D)J recombination[86]. Another illustration is that DSBs if repaired by alternative a-NHEJ pathway instead of the precise NHEJ pathway, will lead eventually to the chromosomal instability due to increase expression of its related proteins[19]. Finally, RAG may preserve some of its transposition activity which is reported to induce mutagenesis, chromosomal instability, and tumorigenesis in various cell lines.

Acute Lymphoblastic Leukemia (ALL)

ALL is a malignant proliferation of B or T lymphocytes in abnormal and uncontrolled manner especially the immature cells and their progenitors. It is the most frequent cancer in pediatrics. The peak of ALL incidences is between (1-4) years, however it also develops in adults. The main cause for ALL is the chromosomal translocations and their consequent genetic abnormalities. These genetic alterations stimulate the expression of protooncogenes and/or disrupt expression of tumor suppressor genes which eventually drive cell growth and tumorigenesis[87].

One of the most frequent translocations in B-cell precursor-ALL (BCP-ALL) is ETV6-RUNX1, also called TEL-AML1 fusion protein. This translocation occurs between chromosome 12 p13 and chromosome 21 p22. This translocation starts in utero where a preleukemic clone is formed in 1 of each 100 healthy newborns[88]. Then, several mutational changes are accumulated to lead eventually to leukemia. Jakobczyk, H stated that ETV6-RUNX and RUNX1 activate RAG1 expression in childhood B-ALL[89]. This aberrant RAG recombinase activity was accused to be the cause for these genomic alterations. The same conclusion was found by Han, Q et al who demonstrated that high RAG expression is correlated with high proliferation markers in B-ALL patients. They correlated the elevated expression of RAG1 with the deletion of IKZF-1 as IKAROS (IKZF1-encoded protein) is RAG expression regulator in leukemic cells by direct binding to its promoter[90].

Another study demonstrated that one percent of TEL-AML1 ALL patients develop leukemia after birth after exposure to infectious antigen (LPS). IL-7 deficiency renders pre-B cells sensitive to LPS. Tsai et al showed that AID and RAG1/2 collaborate in developing chromosomal translocations in human B-cells malignancies. A clinical trial was performed in children with high risk of pre-ALL (with REL-AML1) and found that the high AID expression at time of diagnosis is indicative of low survival rate and high relapse of leukemia. In addition, they injected TEL-AML1 pre-B cells subjected to IL7 withdrawal and exposed to LPS into wildtype mice, mice with either $RAG1^{-/-}$ or $AID^{-/-}$ knockout and double knockout $RAG1^{-/-}$ $AID^{-/-}$mice. They found that all wildtype mice developed leukemia while mice deficient in RAG1 or AID failed to develop overt leukemia[91]. Another study, RAG1/2 together with the DNA crosslinking have an oncogenic role in B-ALL with DUX4/IgH (double homebox4/Immunoglobulin H)[92].

RAG 1 role is not inclined to B-All, it extends to include T-cell ALL. Genome wide analysis was performed by Dong, Y et al and found that Notch1 transcriptional complex stimulate RAG1/2 in primary DN T-cells and in T-ALL[93]. Notch1 is the first and the most important member of the 4 isoforms of transmembrane proteins that upon activation convert to nuclear transcription factor regulating the T-cell developmental process. All these mentioned studies proved the involvement of RAG dysregulation in the development of childhood/adult, B/T- ALL.

Chronic Myeloid Leukemia (CML)

CML is a type of myeloproliferative neoplasm representing 15% of newly diagnosed adult patients with leukemia[94]. Ninety percent of CML patients have BCR-ABL1 fusion protein which is formed by translocation of breakpoint cluster region (BCR) on chromosome (9) and Abelson 1 (ABL1) on chromosome (22). BCR-ABL1 has a tyrosine kinase activity which promotes cell growth and activates signaling pathways leading eventually to development of leukemia[95]. CML has three phases: chronic phase (CP) with insidious symptoms, accelerated phase (AP) and blast phase (BP). Each phase is characterized by a certain range of blast cells (immature cells): lower than 2% in CP, lower than 20% in AP and higher percentages in BP. There are two types of BP: lymphoid (LBP) and myeloid (MBP; more common). Tyrosine kinase inhibitors (TKIs) have achieved success in improving the overall survival and complete remission in CML patients[94,96&97], however patients with BP experience resistance to TKI[98]. Recent study has shown that RAG1 is upregulated in most LBP patients with genetic deletions especially in IKZF2 gene. And DNTT, another enzyme involved in the NHEJP, was elevated in CP patients who then developed LBP. They recommended RAG1 and DNTT as prognostic markers especially in LBP[99]. In mice, RUNX1

in co-operation with RAG/AID induced mutagenesis. Recently Awad, SS found that RUNX1 mutations are one of the most accompanying chromosomal abnormalities in BP-CML and they are commonly found with RAG1 elevation[100]. Yuan, M et al tested the effect of RAG on the cell survival and resistance to TKI in leukemic cell lines. They found that cells with both wildtype RAG and BCR-ABL-1 have repaired DSB with the a-NHEJ pathway and lessen sensitivity to TKI by inducing BCR-ABL1 signaling pathways regardless the level of BCR-ABL1 transcript and the mutation in TK domain[101].

Myelodysplastic Syndrome (MDS)

MDS is a heterogeneous group of malignant hematological disease affecting the HSC. Bioinformatics analysis for data in gene expression omnibus database in addition to clinical trial for confirmation were performed in a recent study. They found that RAG1 low expression (not high as in leukemia) in MDS is associated with bad prognosis, lower survival rate and conversion to acute myeloid leukemia (AML). They found that RAG1 expression is significantly decreased in MDS patients than in healthy control group and RAG1 expression in secondary AML is lower than that in the newly diagnosed candidates. They concluded that RAG1 expression can be used as a prognostic marker in MDS and they recommended assessment of RAG1 in primary AML and increase the sample size of the trial[102].

Lymphoma

Lymphoma is a broad group of malignant lymphocytic neoplasms mainly including Hodgkin and non-Hodgkin lymphoma and more than 90 subtypes. The affected organs are the lymphatic tissue, the bone marrow and it can extend to extra nodal sites[103].

In 1994, a study was conducted to detect the role of RAG1/2 and found that damage of either gene cause stoppage for the maturation process of B and T cells at a very early stage[104]. They considered RAG expression as a marker for detecting the maturation status in leukemia and lymphoma. For example, lower RAG expression in Hodgkin lymphoma with reed-sternberg cells is agreed with the mature status of these type of cells[105].

Other study found that RAG1 (alone without AID) deficiency accelerate the tumorigenesis in mice with Eμ c-myc (chromosomal translocation produced by fusion of c-myc with Igμ)[106]. It seems that RAG expression in lymphoma differs according to the maturation status of cells and the type of chromosomal translocation. More recent study showed that off-target binding of RAG induces lymphoma in Tp53 deficient mice[107].

Conclusion

RAG1 mutations are one of the most frequent causes of primary immunodeficiencies. Additionally, dysregulation of *RAG1* expression might contribute to several hematologic malignancies. All these findings agreed with the indispensable role of RAG1 and V(D)J recombination.

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الجين المحفز لاعادة الارتباط١ حجر الزاوية فى امراض مناعية عديدة وسرطانات

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الجين المحفز لاعادة الارتباط–١ له دور بالغ الاهمية فى عملية اعادة الارتباط بين قطع فى ودى وجى V(D)J recombination بمساعدة الجين المحفز لاعادة الارتباط–٢ هذه العملية لا يمكن الاستغناء عنها لانتاج عدد لا نهائى من الاجسام المضادة Ig و مستقبلات الخلايا التائية TCR وبذلك تكون قادرة على المقاومة والدفاع عن الجسم ضد اى جسم غريب او عدوى.

الطفرات فى هذا الجين تلعب دور كبير فى ظهور امراض نقص المناعة الاولية فهى تظهر على الطفل بعد الولادة بفترة قليلة منها مرض نقص المناعة الشديد والمعقد والذى قد يسبب الوفاء بسبب فرص اصابة الطفل بانواع شديدة الخطورة من العدوى لغياب الخلايا المناعية. وهناك امراض اخر تظهر فى الاطفال الاكبر سنا مع حدوث اعراض اقل خطورة ولكن مع وجود بعض تأثيرات المناعة الذاتية.

وقد وجد ان خلل التعبير الجينى لهذا الجين يلعب دورا فى حدوث سرطانات الدم منها اللوكيميا الحادة و المزمنة. فى هذا البحث نستعرض الدور الذى يلعبه هذا الجين فى الامراض المختلفة وأليه حدوث هذه الامراض.