Experimental Hematology

Experimental Hematology 2020;90:30–38

INVITED REVIEW

The critical role of germinal center-associated nuclear protein in cell biology, immunohematology, and hematolymphoid oncogenesis

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(Received 11 June 2020; revised 14 August 2020; accepted 16 August 2020)

Germinal center-associated nuclear protein (GANP) is a unique and multifunctional protein that plays a critical role in cell biology, neurodegenerative disorders, immunohematology, and oncogenesis. GANP is an orthologue of *Saccharomyces* Sac3, one of the components of the transcription export 2 (TREX-2) complex and a messenger RNA (mRNA) nuclear export factor. GANP is widely conserved in all mammals, including humans. Although GANP was originally discovered as a molecule upregulated in the germinal centers of secondary lymphoid follicles in peripheral lymphoid organs, it is expressed ubiquitously in many tissues. It serves numerous functions, including making up part of the mammalian TREX-2 complex; mRNA nuclear export via nuclear pores; prevention of R-loop formation, genomic instability, and hyper-recombination; and B-cell affinity maturation. In this review, we first overview the extensive analyses that have revealed the basic functions of GANP and its ancestor molecule Sac3, including mRNA nuclear export and regulation of R-loop formation. We then describe how aberrant expression of GANP is significantly associated with cancer development. Moreover, we discuss a crucial role for GANP in B-cell development, especially affinity maturation in germinal centers. Finally, we illustrate that overexpression of GANP in B cells leads to lymphomagenesis resembling Hodgkin lymphoma derived from germinal center B cells, and that GANP may be involved in transdifferentiation of B cells to macrophages, which strongly affects Hodgkin lymphomagenesis.

Germinal center-associated nuclear protein (GANP) is a unique and amazing molecule that has numerous cell biological, neurodegenerative, immunohematological, and oncogenic roles, and is critical for human health.

We discovered GANP in 2000 as a molecule associated with mouse B-cell differentiation and affinity maturation in germinal centers into secondary lymphoid follicles; in this context, somatic hypermutation of *variable-region* genes and class switching of B-cell receptors (BCRs) occur in B cells driven against T cell-dependent antigens [1,2]. We immunohistochemically screened monoclonal antibodies that recognized factors expressed on germinal center B cells and found that one antibody, designated 29–15, reacted to a molecule that was upregulated in germinal centers. Using a λgt11 expression cloning method, we finally identified a novel mouse gene, named *ganp*. Mouse GANP is a 210-kDa nuclear protein composed of 1,971 amino acids (aa), and the middle portion of mouse GANP bears sequence similarity to *Saccharomyces cerevisiae* Sac3 (1,301 aa; 23% at amino acid level).

It is now well known that orthologues of yeast Sac3 are widely conserved in all mammals, including *Homo sapiens,* where it has come to be known as GANP

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0301-472X/
https://doi.org/10.1016/j.exphem.2020.08.007

(Figure 1). Human *ganp* is located on a chromosome 21q22.3 locus and encodes a 1,980-aa protein [2]. In addition to a Sac3 conservative domain, mammalian GANP contains two domains that share sequence similarity with two other mammalian proteins. One domain is approximately 150 aa long and is located on the amino (N)-terminal side of the Sac3 conservative domain, which bears similarity to DNA primase p49 [3]. The other domain is MCM3AP (MCM3-acetylating protein), which resembles the carboxyl (C)-terminal region of human and mouse GANP [4,5]. We suggest that MCM3AP may be a short form of GANP produced by alternative splicing, although another group reported that *mcm3ap* was transcribed from a unique promoter (see Figure 1) [6]. Confusingly, some research groups use the gene name *mcm3ap* to refer to the full-length form of *ganp*, rather than the short form. Historically, human MCM3AP has been reported as a novel 80-kDa protein encoded by *map80* [4]. Afterward, the gene symbol was altered from *map80* to *mcm3ap* based on its intrinsic activity as an acetylase [5]. Although the gene name *mcm3ap* firstly registered was annotated in several databases, many researchers, including us, insist that GANP (210-kDa protein) is a long form of MCM3AP (80-kDa protein). Moreover, the roles and functions of the GANP protein are obviously different from those of MCM3AP [1,2]. To clear up the confusion, *ganp* is preferable as the name of the gene that encodes GANP.

Over the last 20 years, it has also been revealed that GANP is expressed ubiquitously in the mammalian body not only to facilitate B-cell affinity maturation of lymphocytes in the context of humoral immunity, but also to serve many functions in many tissues. In this review, we focus on the biological significance of GANP, which is especially important for messenger RNA (mRNA) nuclear export, immunohematology of B-cell differentiation, and oncogenesis of hematolymphoid cells.

Functions of GANP and its related molecules

It is widely accepted that GANP plays numerous roles, including those that we have already discovered and those that we predict, with impacts in cell biology, neurology, immunohematology, and oncology. Here we summarize GANP's many functions as clearly and comprehensively as possible. In particular, the important hematologic functions of GANP, such as B-cell affinity maturation and B-cell transdifferentiation into macrophages, are discussed later.

Composition of the TREX-2 complex

In 1988, Novick et al. [7] discovered Sac3 (suppressor of actin) in *S. cerevisiae*, and found that mutant Sac3 decreased the temperature sensitivity of actin gene expression in the *act1-1* mutant of this yeast. However, subsequent studies reported that *sac3*-deficient, *act1-1* mutant *S. cerevisiae* did not exhibit decreased

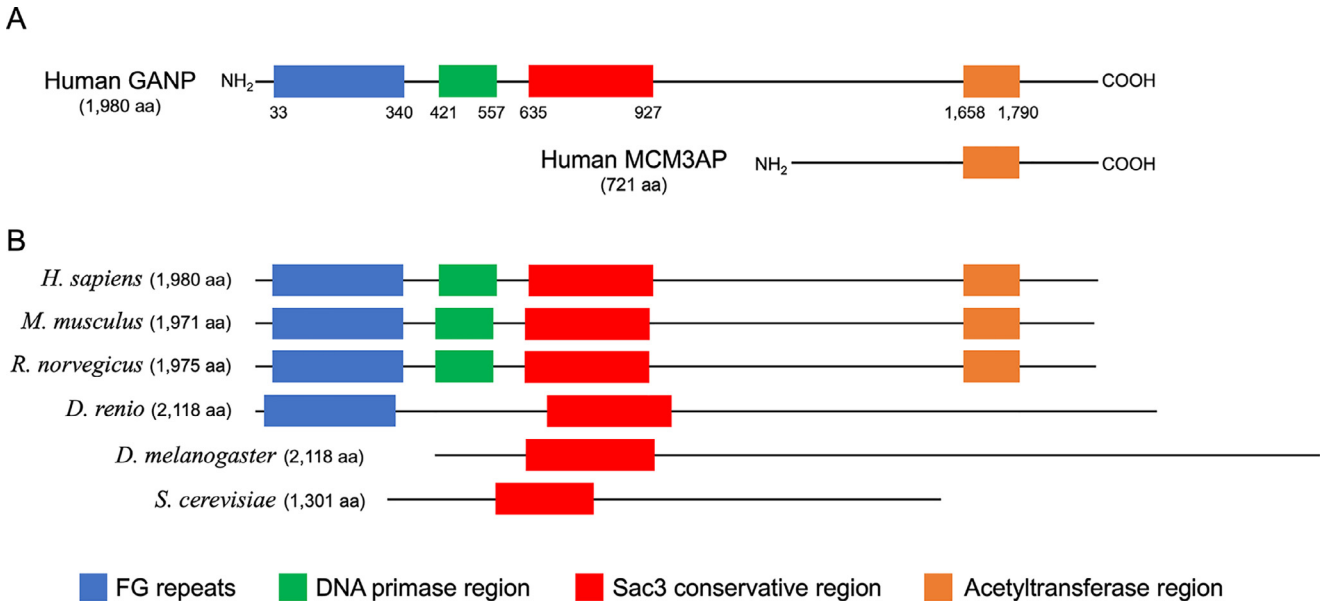

Figure 1. Molecular structures of Sac3/GANP family proteins in several species. **(A)** Human GANP is a 1,980-aa protein that consists of several functional domains, including phenylalanine–glycine (FG) repeats, a DNA primase region, a Sac3 conservative region, and an acetyltransferase region. The C-terminal region of GANP is designated MCM3AP (MCM3-acetylating protein); this short form has been detected only in humans. **(B)** Structures of GANP orthologues compared among several species. The DNA primase and acetyltransferase region are detected only in higher eukaryotes. The bottom protein with 1,301 aa from *Saccharomyces cerevisiae* corresponds to Sac3.

temperature sensitivity of actin gene expression, suggesting that reduction of temperature sensitivity is not simply caused by loss-of-function Sac3 [8]. At that point, the molecular functions of Sac3 were unknown, although it had been revealed that *Sac3*-deficient yeast exhibited a cell cycle mitotic delay and chromosome instability [9].

The turning point came in 2002 when Fischer et al. [10] found that Sac3 forms a stable complex with Thp1 and functions in transcription elongation. Interestingly, Sac3 or Thp1 mutation causes a severe defect in mRNA nuclear export. In addition, Sus1, a component of the SAGA complex with histone acetylase activity; Cdc31, a yeast centrin; and Sem1, a component of the ubiquitin/proteasome system, were similarly indispensable for mRNA nuclear export [11–13]. Together with these results, it was eventually revealed that Thp1, Sus1, Cdc31, and Sem1 bind to a Sac3 scaffold and form a complex molecule involved in mRNA nuclear export and transcription-coupled DNA recombination. They named the complex of Sac3, Thp1, Sus1, Cdc31, and Sem1 the transcription-export 2 (TREX-2) complex. We should note that 3′ repair exonuclease 2 (TREX2) is a completely different single small protein, and indeed, we have seen descriptions in some papers that "TREX-2 complex" stands for "3′ repair exonuclease-2 complex"; however, this is incorrect.

It is now understood, as described above, that the protein sequence of *Saccharomyces* Sac3 is homologous to that of the middle portion of GANP and is evolutionally conserved in sequence and function among several species. In addition, the mammalian TREX-2 complex is thought to have a similar function in mRNA nuclear export. The mammalian TREX-2 complex is composed of GANP, PCID2, ENY2, centrin3/4, and DSS1 (the corresponding orthologues of *Saccharomyces* spp. are Sac3, Thp1, Sus1, Cdc31, and Sem1, respectively).

GANP-dependent mRNA nuclear export via nuclear pores

The TREX-2 complex stably co-localizes with nuclear pore complexes at the nuclear periphery and plays an important role in mRNA nuclear export through the nuclear pore by interacting with NXF1 [14]. NXF1 (mammalian orthologue of *Saccharomyces* Mex67) is a nuclear RNA export factor and includes two important domains: one is an RNA-binding domain (RNA recognition motif and leucine-rich repeats) that facilitates association of NXF1 with mRNA to form a messenger ribonucleoprotein (mRNP), and the other is an ubiquitin-associated–like domain that mediates interactions with phenylalanine–glycine (FG) repeat motifs. Because the N-terminal side of GANP contains FG repeat motifs, nuclear pore-localized TREX-2 complex can associate with NXF1-binding mRNPs. Thus, many researchers now accept that GANP is an integral component of the mammalian mRNA nuclear export machinery and facilitates transfer of NXF1-binding mRNPs to nuclear pore complexes [15].

In mammals, however, researchers also believe there exist both GANP-dependent and GANP-independent pathways for transfer of NXF1-binding mRNPs to nuclear pore complexes, and we were the first to recognize this fact. We found that *ganp* RNA interference (RNAi) in HeLa cells led to cell cycle arrest at the G2/M phase, increased abnormal chromosome alignment of metaphase chromosomes, and finally led to cell apoptosis. These defects followed destabilization of cohesin, a centromeric protein complex that mediates sister chromatid cohesion, caused by a defect in mRNA nuclear export and erroneous translation of shugoshin-1, a molecule that protects centromeric cohesion [16]. On the other hand, we found that RNAi of not *ganp* but *pcid2* also resulted in a cell cycle abnormality, increased apoptosis, and polyploidy, which were caused by reduced translation of Mad2, a spindle checkpoint protein [17]. Based on these results and those from other studies, all transcripts can be separated into GANP/NFX1-dependent and only NFX1-dependent ones. The former may include specific classes of mRNAs, such as RNA synthesis and processing factors that may enable rapid changes in gene expression [18].

Prevention of R-loop formation, genomic instability, and hyper-recombination

From 2001 to 2003, Aguilera and colleagues [19–21] made revolutionary breakthroughs in *S. cerevisiae*. Not only did they announce that *S. cerevisiae* deficient in Sac3 or Thp1 exhibit a hyper-recombination phenotype, but they unraveled the mysterious relationship between transcription and recombination during mRNA metabolism by invoking a DNA:RNA hybrid model [19–21]. When mRNA metabolism, especially mRNA nuclear export, is disturbed, mRNA that cannot move out of the nucleus forms a hybrid with one of two strands of DNA and disturbs the other DNA strand. The DNA:RNA hybrid and the dispersed single DNA strand form an "R-loop" structure that easily results in DNA breaks, genomic instability, and hyper-recombination. The DNA breaks that are induced by R-loop formation, which result from inhibited mRNA nuclear export, are called "transcription-coupled DNA damage."

We now know that GANP prevents hyper-recombination in mammals. To establish this fact at the time, we created immortalized mouse embryonic fibroblasts (MEFs) from *ganp*-heterodeficient mice ($ganp^{+/d}$ MEFs). $ganp^{+/d}$ MEFs exhibited a marked increase in DNA recombination compared with wild-type MEFs, whereas spontaneous DNA recombination was

overwhelmingly inhibited by forced overexpression of GANP in NIH3T3 cells. We additionally showed in another experiment that GANP also suppressed DNA recombination in *aid*-induced NIH3T3 cells, in which activation-induced cytidine deaminase (AID) frequently introduced DNA mutations by changing a cytosine:guanine (C:G) base pair into a uracil:guanine (U:G) mismatch. In summary, the Sac3 conservative domain in GANP can suppress hyper-recombination and contribute to genomic stability in mammalian cells [22].

Prevention of cancerization

Although little is known about the relationship between oncogenesis and GANP, one of the reasons for oncogenesis may be impairment of mRNA nuclear export machinery caused by *ganp* dysfunction. Because some mRNA nuclear export is dependent on GANP, as described above, *ganp* dysfunction may dysregulate some pivotal proteins, such as shugoshin-1, which may affect oncogenesis in various cases. Oncogenesis is also caused by R-loop formations, which induce hyper-recombination and genomic instability.

It has recently been reported that mRNAs are transported from the nucleus to the cytoplasm through two pathways: a bulk export pathway involving NXF1 (described above in detail) and a specialized pathway involving chromosome region maintenance 1 (CRM1) [23]. Although it is still controversial whether dysregulation of the mRNA nuclear export machinery directly drives cancer initiation, it is well known that a defect in the specialized mRNA export pathway is frequently observed in various cancers [24]. CRM1 is reportedly overexpressed in a variety of human neoplasms, such as endocervical carcinomas of the uterus and gliomas of the central nervous system, and its overexpression is closely correlated with patient prognosis [25,26]. Abnormal expression of nucleoporins (Nups) is also detected in cancers; for example, a t(6;9)(p23;q34.1); *dek–nup214* chromosomal translocation is observed in a subtype of acute myeloid leukemia [27].

Recently it has been revealed that dysfunction of the bulk mRNA export pathway is also linked to cancer. As described above, the TREX-2 complex facilitates transfer of NXF1-binding mRNPs to nuclear pore complexes and plays a critical role in the bulk mRNA export pathway. GANP, a component of the TREX-2 complex, is frequently over- or underexpressed in various tumors. Overexpression of GANP in tumor cells was observed in lymphomas (see below for details), malignant melanomas, and liver fluke-associated cholangiocarcinomas [28–30], whereas underexpression was observed in breast cancers and glioblastomas [31,32]. In the case of breast cancers, we revealed that GANP deficiency is strongly associated with mammary carcinogenesis. Breast cancers frequently occur in two *ganp* mutant mouse lines, female *ganp*-heterodeficient mice and mammary-specific *ganp*-deficient mice. The latter were *ganp* floxed (fl) (*wap–cre–ganp*$^{fl/fl}$) mice, in which a part of *ganp* was eliminated between two *loxP* sequences inserted in the 5′-flanking region of exon 2 and the 3′-flanking region of exon 3 using cre/loxP recombination technology. With this method, *ganp* excision occurred when the *wap* promotor was activated on the 18th day of pregnancy, *cre* was subsequently translated, and the *loxP* DNA sequences were cleaved. After the 18th day of pregnancy, the mammary glands in these mice gradually manifested structural atypia and finally became cancerous. Together with in vitro experiments, these results indicate that GANP can suppress the DNA damage induced by estrogen exposure and has an anti-oncogenic effect on breast carcinogenesis [31].

Nuclear import of MCM3 and regulation of cell cycle

As described above, the C-terminal region of human and mouse GANP is identical to MCM3AP (MCM3-acetylating protein). MCM3AP is a MCM3-binding molecule that acetylates MCM3 to suppress cell cycle progression and facilitates nuclear import of MCM3 [4,5].

Pathogenesis of Charcot–Marie–Tooth disease

It has been reported that mutations in *ganp* were detected in nine patients and five unrelated families with Charcot–Marie–Tooth disease [33]. Charcot–Marie–Tooth disease is a hereditary neuropathy of the peripheral nervous system characterized by progressive loss of muscle and touch sensation. More than 40 genes have been reported as responsible for Charcot–Marie–Tooth disease, and in 2017, it was discovered that a *ganp* mutation also causes a variant of this disease [33]. The variant of the disease presents in children, who develop axonal or demyelinating neuropathy; these patients may carry either heterozygous or homozygous mutant *ganp* alleles. However, according to research using patient fibroblasts, this mutant form of GANP localized normally to the nuclear envelope, and severe GANP dysfunction did not affect DNA repair in vitro, even in patients with a nonsense mutation in *ganp*. Thus, a role for GANP in the patho-etiology of Charcot–Marie–Tooth disease is still unclear.

DNA primase activity resembling p49

As described above, human GANP and mouse GANP contain a 150-aa-long domain on the N-terminal side of the Sac3 conservative domain that is slightly homologous in protein sequence to the DNA primase p49 [3]. In vitro analysis has revealed that recombinant GANP protein synthesizes RNA primers, resulting in DNA replication in the presence of DNA polymerase I and

single-stranded DNA templates [3]. The DNA primase activity of GANP is controlled by phosphorylation at Ser^{502} (putative phosphorylation site by Cdk2) and induced by CD40-mediated signaling in vitro or by antigen stimulation in vivo [3]. It has recently been revealed that Ser^{502}-phosphorylated GANP is preferentially detected in germinal center B cells. In addition to the conventional DNA polymerase α-primase complex, DNA primase in GANP may be essential for excess DNA synthesis to promote somatic hypermutation and affinity maturation in germinal center B cells.

Immunohematological relationship between GANP and B-cell affinity maturation

Affinity maturation is now thought to be composed of two steps, somatic hypermutation and clonal selection, which occur in the germinal centers of secondary lymphoid follicles [34]. Somatic hypermutation is a programmed process for introducing mutations and affects *IgV*, a part of the antigen-binding coding sequences of *Ig* genes. The mutations promote the diversity of antibodies by altering their binding specificity and affinities. Clonal selection is the process by which B cells that react with no antigens or some antigens only very weakly are eliminated; these defective B cells are selected and made to undergo apoptosis by follicular helper T cells in the germinal centers. It is now known that GANP is an essential immunohematological molecule that maintains immune function by playing an important role in somatic hypermutation and probably in clonal selection of antigen-specific B cells that express high-affinity BCRs.

Germinal center formation and somatic hypermutation

The functions of GANP during germinal center formation and affinity maturation have been clarified using laboratory mouse models [35]. Unfortunately, however, *ganp*-homodeficient mice manifested embryonic lethality by around embryonic day 11.5, presumably caused by abnormal morphogenesis of the brain and heart (unpublished data). In addition, it was difficult to establish a hematopoietic system–specific *ganp*-deficient mouse that was also engineered to reconstitute *ganp*-deficient fetal liver cells into immunocompromised mice, as very few liver cells could be obtained from homozygous embryos.

Hence, we established B cell–specific *ganp*-deficient ($cd19\text{–}cre\text{–}ganp^{fl/fl}$) mice using cre/loxP recombination technology [35]. Compared with wild-type mice, $cd19\text{-}cre\text{-}ganp^{fl/fl}$ mice exhibited normal B-cell number, development, and subpopulations; serum Ig levels;mitogen-induced B-cell proliferation in vitro; immune responses against T cell–independent antigen; and B-cell class switching, whereas germinal center formation was retarded even on immunization with T cell–dependent antigen. Moreover, somatic mutations in *IgV* and high-affinity variant induction ($^{33}Trp \rightarrow {}^{33}Leu$) of the $V_H186.2$ region occurred less frequently in $cd19\text{–}cre\text{–}ganp^{fl/fl}$ mice than that in wild-type mice as a response to immune reaction of a hapten of nitrophenyl-chicken γ-globulin [35].

We also established and analyzed *ganp* transgenic C57BL/6 ($Ig\text{-}ganp^{Tg}$) mice, in which GANP was overexpressed in B cells [36]. $Ig\text{-}ganp^{Tg}$ mice had a normal phenotype in terms of B-cell number, development, and subpopulations; serum Ig levels; and immune responses against T cell–independent antigens; however, antibodies against a hapten of nitrophenyl exhibited much higher affinity in $Ig\text{-}ganp^{Tg}$ mice than they did in wild-type C57BL/6 mice. $Ig\text{-}ganp^{Tg}$ mice were also able to produce extremely high-affinity anti-human immunodeficiency virus-1 monoclonal antibodies with neutralizing activity. These results clearly indicated that GANP is essential for production or maintenance of high-affinity B cells in germinal centers.

It has been reported that GANP may be essential for transporting AID to B-cell nuclei for targeting *IgV* [37]. However, whether GANP deficiency leads to impairment of somatic hypermutation remains controversial.

Double-strand breaks at immunoglobulin variable regions

Furthermore, using ligation-mediated polymerase chain reaction, we found that double-strand breaks at *IgV* were more frequently observed in $Ig\text{-}ganp^{Tg}$ B cells and less frequently observed in *ganp*-deficient B cells, compared with control B cells [38]. These results suggested that the number of *IgV* double-strand breaks in germinal center B cells is positively correlated with GANP expression. Double-strand breaks at *IgV* induced by GANP may be required for affinity maturation, and lack of GANP may cause impaired affinity maturation of antigen-driven B cells and subsequently trigger B-cell apoptosis as clonal selection controlled by follicular dendritic cells. Note that GANP induces double-strand breaks only at *IgV* and, opposingly, avoids transcription-coupled DNA damage in the other genome. It is speculated that double-strand DNA breaks at *IgV* are phenomenologically unrelated to mRNA nuclear export, R-loop formation, and DNA primase activity; however, this idea remains controversial.

Modulation of GANP expression by the upstream molecule Lyn

GANP is a molecule located downstream of Lyn, an src-type tyrosine kinase [39]. Lyn plays a role in signal transduction of intracytoplasmic molecules (such as surface IgM [sIgM] and CD40) in B cells in the peripheral lymphoid organs [39]. Lyn-deficient ($lyn^{-/-}$) mice have impaired development of germinal centers in the

spleen and decreased antibody affinity, and GANP expression is decreased in Lyn-deficient chicken DT40 cells and mouse B cells [40]. These results suggest that Lyn may control formation and proliferation of germinal centers via GANP. Moreover, both *Ig-ganp*^{Tg}*lyn*$^{-/-}$ mice and *lyn*$^{-/-}$ mice exhibited similar B-cell differentiation, serum Ig levels, and impaired germinal center formation phenotypes, although mature B cells, similar to germinal center B cells, were partly rescued and affinity maturation was potentially recovered in *Ig-ganp*^{Tg}*lyn*$^{-/-}$ mice [41]. These results indicate that GANP is indispensable for germinal center formation, but plays a critical role in Lyn-mediated signaling for the clonal selection of B cells in peripheral lymphoid organs (Figure 2A).

Hodgkin lymphomagenesis and transdifferentiation between B cells and macrophages

Transdifferentiation, also known as reprogramming, is the process by which a differentiated somatic cell transforms into another type of differentiated cell without undergoing an intermediate pluripotent state or dedifferentiating into a progenitor cell.

Hodgkin lymphoma is a lymphoid neoplasm characterized by the development of large dysplastic lymphocytes called Hodgkin and Reed–Sternberg cells, which originate from B cells located in the germinal centers of peripheral lymphoid organs and may or may not be associated with Epstein–Barr virus infection. Interestingly, Hodgkin lymphoma cells exhibit altered characteristics, such as partial loss of B-cell lineage markers, including surface IgM (sIgM), CD20, and CD79a, and sometimes express biphenotypic characteristics of B cells and macrophages, such as phagocytic activity and macrophage-derived cytokine secretion [42].

Our research using *lyn*$^{-/-}$ and *Ig-ganp*^{Tg} mice indicated that GANP regulates cell transdifferentiation between B cells and macrophages [42]. Moreover, GANP overexpression may invoke Hodgkin lymphoma, which exhibits a B-cell/macrophage biphenotype [42].

Transdifferentiational B-cell/macrophage biphenotypic cells in lyn$^{-/-}$*mice*

B-Cell/macrophage biphenotypic cells, which express both Ig and macrophage-specific marker CD11b, appear in increased numbers in the spleens of *lyn*$^{-/-}$ mice compared with those in *lyn*$^{+/-}$ mice [39,42].

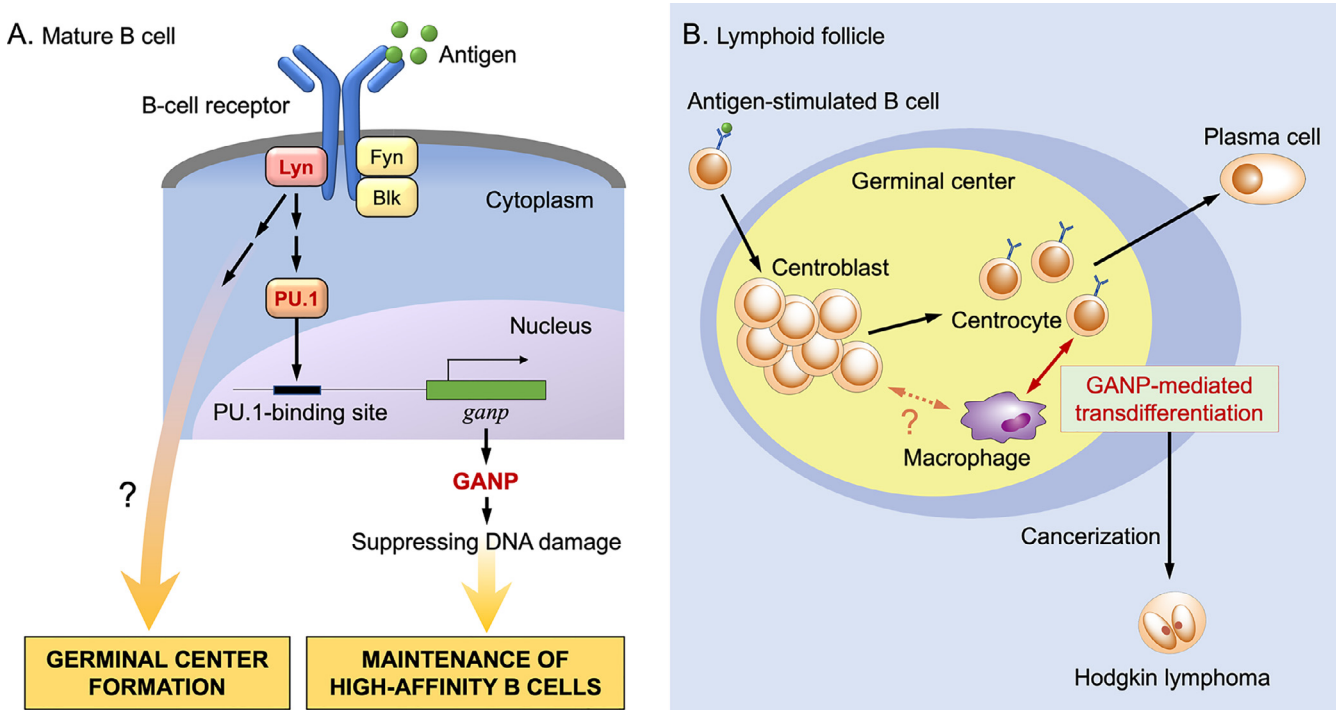

Figure 2. Schematic diagrams of the role of GANP during B-cell maturation and differentiation. **(A)** Antigen stimulation induces tyrosine phosphorylation of src-family kinases, including Lyn, followed by activation of downstream molecules. The mouse *ganp* promoter region contains a PU.1-binding site, and GANP expression is regulated by Lyn through PU.1. The introduction of the *ganp* gene into *lyn*$^{-/-}$ mice did not restore germinal center formation; however, the number of germinal center-like B cells with high-affinity antibodies was partly rescued out of the germinal center. **(B)** Antigen-stimulated B cells rapidly proliferate in germinal centers and become centroblasts, in which B-cell receptor is downregulated. Afterwards, centroblasts differentiate into centrocytes, which are subject to affinity maturation with help from follicular dendritic cells and follicular helper T cells. Hodgkinoid lymphoma cells developed in *Ig-ganp*^{Tg} mice exhibit a B-cell/macrophage biphenotype, an intermediate state between B cell and macrophage.

Approximately one-third of $CD11b^+$ cells in the spleens of 14-week-old $lyn^{-/-}$ mice were cytoplasmic IgM (cIgM) positive, although $cIgM^+CD11b^+$ cells were still observed less frequently in 8-week-old $lyn^{-/-}$ mice [42]. Furthermore, in *Ig-ganp*^{Tg}$lyn^{-/-}$ mice, the cell population of $cIgM^+CD11b^+$ cells in the spleen was almost normal [42]. Thus, biphenotypic $cIgM^+CD11b^+$ cells were mostly observed in $lyn^{-/-}$ mice but not in control or *Ig-ganp*^{Tg}$lyn^{-/-}$ mice, which indicates that the biphenotypic cells may be B cells incompletely transdifferentiated to macrophages by lack of GANP and that GANP regulates cell transdifferentiation from B cells to macrophages in a Lyn-independent manner.

Hodgkinoid lymphomas in Ig-ganp^{Tg} mice

B-Cell lymphomas frequently develop in the livers and spleens of *Ig-ganp*^{Tg} mice after prolonged observation [42]. They exhibited abnormally large and irregularly shaped B cells. Astonishingly, these cells exhibited B-cell/macrophage biphenotypic hodgkinoid characteristics that resemble human Hodgkin lymphoma. In addition, although biphenotypic hodgkinoid cells were originated from B cells expressing rearranged μ-heavy/κ-light chains and cytoplasmic CD45R (B220), they not only eliminated sIgM and surface CD45R(B220) but also acquired macrophage-specific characteristics, such as major histocompatibility complex class II, F4/80, CD68, and CD204 [28]. Moreover, the hodgkinoid cell line established from *Ig-ganp*^{Tg} mice exhibited high phagocytotic activity in vitro and secreted both cytokines (granulocyte/macrophage colony-stimulating factor [GM-CSF], macrophage colony-stimulating factor [M-CSF], interleukin [IL]-4, IL-10, IL-12, IL-13, tumor necrosis factor [TNF]-α, vascular endothelial growth factor, and thrombopoietin) and CC chemokines (monocyte chemoattractant protein [MCP]-1, MCP-5, keratinocyte chemoattractant, and RANTES) [42]. Collectively, these findings suggest that these hodgkinoid cells exhibit the functions of macrophages and that GANP may be associated with Hodgkin lymphomagenesis and transdifferentiation from B cells to macrophages.

The *Ig-ganp*^{Tg} mouse, a novel cytological model of Hodgkin lymphoma, is quietly beneficial. Approximately half of human Hodgkin lymphoma cases are caused by Epstein–Barr virus (EBV) infection; however, EBV cannot infect mice and immortalize B cells [43]. GANP is known to operate downstream of CD40, and CD40 activates NF-κB signaling constitutively in Hodgkin and Reed–Sternberg cells. *Ig-ganp*^{Tg} mice are the only mice in which this signaling cascade may be critical for Hodgkin lymphomagenesis. Further studies using *Ig-ganp*^{Tg} mice are required to fully elucidate the mechanisms of Hodgkin lymphomagenesis.

Hodgkin lymphomas in humans

GANP is overexpressed in human Hodgkin lymphoma cells, as well as Hodgkinoid lymphoma cells developed in *Ig-ganp*^{Tg} mice [42]. Because GANP overexpression is closely related to oncogenesis of Hodgkin lymphoma, which is derived from germinal center B cells, GANP plays a critical role not only in affinity maturation of B cells but also in transdifferentiation from B cells to macrophages in the germinal centers, even in humans (Figure 2B). In addition, the upstream or downstream molecules of GANP may conceivably be alternative therapeutic targets of human Hodgkin lymphoma.

Conclusion

GANP has a structure homologous to that of yeast Sac3 and is a member of the TREX-2 complex. It has been documented as a critical regulator of transcription-coupled DNA damage associated with mRNA metabolism. We originally identified GANP as a molecule that is upregulated in the germinal centers of peripheral lymphoid organs and have focused on its functional role in the immunohematological field using in vivo studies of mutant mice for two decades. GANP functions can be partly explained by transcription-coupled DNA damage; however, its complex in vivo functions are still not fully understood, as the *ganp* gene contains many functional domains and exhibits a complicated array of interactions with other molecules. As a consequence, it may be difficult to further study GANP like other macromolecules.

In this review, we also introduced a role for GANP in modulating oncogenesis. Using mouse models, we showed that GANP expression is not simply enhanced in various tumors, but may also be directly or indirectly involved in driving oncogenesis. Why GANP is overexpressed or underexpressed, depending on tumor histological type, remains an important unanswered question. It is crucial that we reveal how GANP differentially affects oncogenesis in hematological malignancies, malignant melanoma, and cholangiocarcinoma in contrast to breast cancers and high-grade gliomas. Further investigations should be conducted to unravel the details of this complex situation.

Acknowledgments

We thank Professor Nobuo Sakaguchi for supervising several research projects concerning GANP. We also thank Professor Makoto Kuroda for encouragement. Submission of the review article was supported by a Grant-in-Aid for Young Scientists 20K16228 from the Japan Society for the Promotion of Science (YS). SP was supported by a scholarship from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Conflict of interest disclosure

The authors have no conflicts of interest to declare.

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