available. A haploidentical relative is a suitable alternative when an HLA-matched sibling is not available.

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G-protein coupled receptor (GPCR) mutations in lymphoid malignancies: linking immune signaling activation and genetic abnormalities

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Marginal-zone B-cell lymphomas of mucosa-associated lymphoid tissue (MALT) arise from a background of chronic microbial infections or autoimmune disorders at diverse extranodal sites.[1,2] The best characterized examples are gastric MALT lymphoma following *Helicobacter pylori* infection, and salivary gland or thyroid MALT lymphomas developing in patients with Sjögren syndrome or Hashimoto thyroiditis, respectively.[3,4] It is now accepted that such chronic microenvironmental inflammation stimulates surface BCR, TLR and CD40 receptors in B lymphocytes that converge to activate downstream NF-κB signaling, which leads to the local expansion of autoreactive B cells eventually suffering malignant transformation through the acquisition of genetic changes.[5] Among them, three hallmark chromosomal translocations, t(11;18)(q21;q21), t(14;18)(q32;q21) and t(1;14)(p22;q32), play a major part in MALT lymphoma origination through dysregulating MALT1 enzymatic activity that constitutively triggers the NF-κB pathway independently of antigenic stimuli.[6-9] Other recurrent mutations in the *MYD88*, *TBL1XR1*, *KLF2* and *TNFAIP3* genes are similarly a consequence of chronic receptor stimulation and further promote NF-κB signaling, contributing to lymphoma transformation.[10] A second signaling pathway recurrently found to be involved in marginal-zone lymphoma (MZL) pathogenesis is NOTCH, primarily including mutations in the C-terminal PEST domain of *NOTCH2* and *NOTCH1* genes that enhance the stability of intracellular protein domains after being triggered by microenvironmental interactions.[5] Thus, both the active chronic immunological stimuli and the acquired genetic abnormalities have critical roles during the development of MALT lymphoma through dysregulating similar molecular mechanisms.

In this issue of the Journal, Moody *et al.* expand this intriguing oncogenic co-operation between immune receptor signaling and genetic abnormalities in MALT lymphoma. They report the discovery of somatic mutations in the G-protein coupled receptors (GPCRs) GPR34 and CCR6 not previously reported in human malignancies.[11] The Authors performed whole exome sequencing of 21 salivary gland and thyroid tumors, and also carried out sequencing analysis of 249 MALT lymphomas, to define distinct mutation profiles in tumors of various sites. Those of the salivary gland were characterized by frequent TBL1XR1 and GPR34 mutations, whereas CCR6 changes were found in MALT lymphomas at different locations. The majority of GPR34 and CCR6 mutations clustered in the cytoplasmic tail, potentially leading to truncated gain-of-function proteins enabling constitutive ligand-dependent receptor activation.[12] Thus, a novel synergistic mechanism between constitutively active NF-κB and GPCR signaling pathways is proposed to participate in the development of MALT lymphoma (Figure 1A).

G-protein coupled receptors are made up of a large superfamily of cell surface ligands that regulate and transmit extracellular signals across the plasma membrane to induce a range of cellular and physiological responses. Despite this diversity, however, their structure, activation, signaling and regulatory mechanisms are remarkably conserved. GPCRs contain seven transmembrane spanning α-helices linked by three intracellular and three extracellular loop regions, an extracellular amino-terminal domain, and an intracellular carboxyl tail. In response to ligand binding, the receptor undergoes conformational changes to couple and activate heterotrimeric G proteins (Gα, Gβ and Gγ) at the plasma membrane that regulate downstream signaling effectors. To turn off the response, GPCR kinases are recruited to phosphorylate the receptor and prepare them for β-arrestin binding, which compete with G protein coupling and desensitize the G-protein-mediated signaling response.[13] Aberrant receptor activity has been shown in

numerous disorders including cancer, ranging from deregulated patterns of expression in particular tumor entities to pathogenic gene mutations potentially contributing to malignant transformation.[14,15] Consequently, GPCR pharmacological targeting is under development for a variety of indications such as inflammation, neurobiological and metabolic disorders, and cancer.[14,16]

The discovery of G-protein coupled receptor 34 (GPR34) mutations in MALT lymphoma is not totally unexpected, as deregulation of the *GPR34* gene through juxtaposition to *IGVH* gene sequences has already been reported after the molecular cloning of a recurrent t(X;14)(p11;q32) chromosomal translocation.[17] Elevated GPR34 expression was detected independently of the translocation in most other MALT lymphoma cases, leading to increased proliferation through constitutive activation of the ERK and NF-κB pathways.[17] In line with this gain-of-function oncogenic potential, the majority of GPR34 mutations identified by Moody *et al.* are nonsense or frameshift changes clustered in the C-terminal region, resulting in truncated proteins that would eliminate or impair a key phosphorylation motif and thus deregulate the receptor desensitization process (Figure 1B).[12] The remaining GPR34 mutations are missense changes including Y327N, which locates in between the transmembrane domain and the cytoplasmic tail, and R84H and D151A at the intracellular loops, which also seem to induce constitutive receptor signaling activation.[12,18] Lysophosphatidylserine, an endogenous lipid mediator generated by the hydrolysis of the membrane phospholipid phosphatidylserine, has been proposed as one of the ligands of GPR34.[18] Because most MALT lymphoma cases

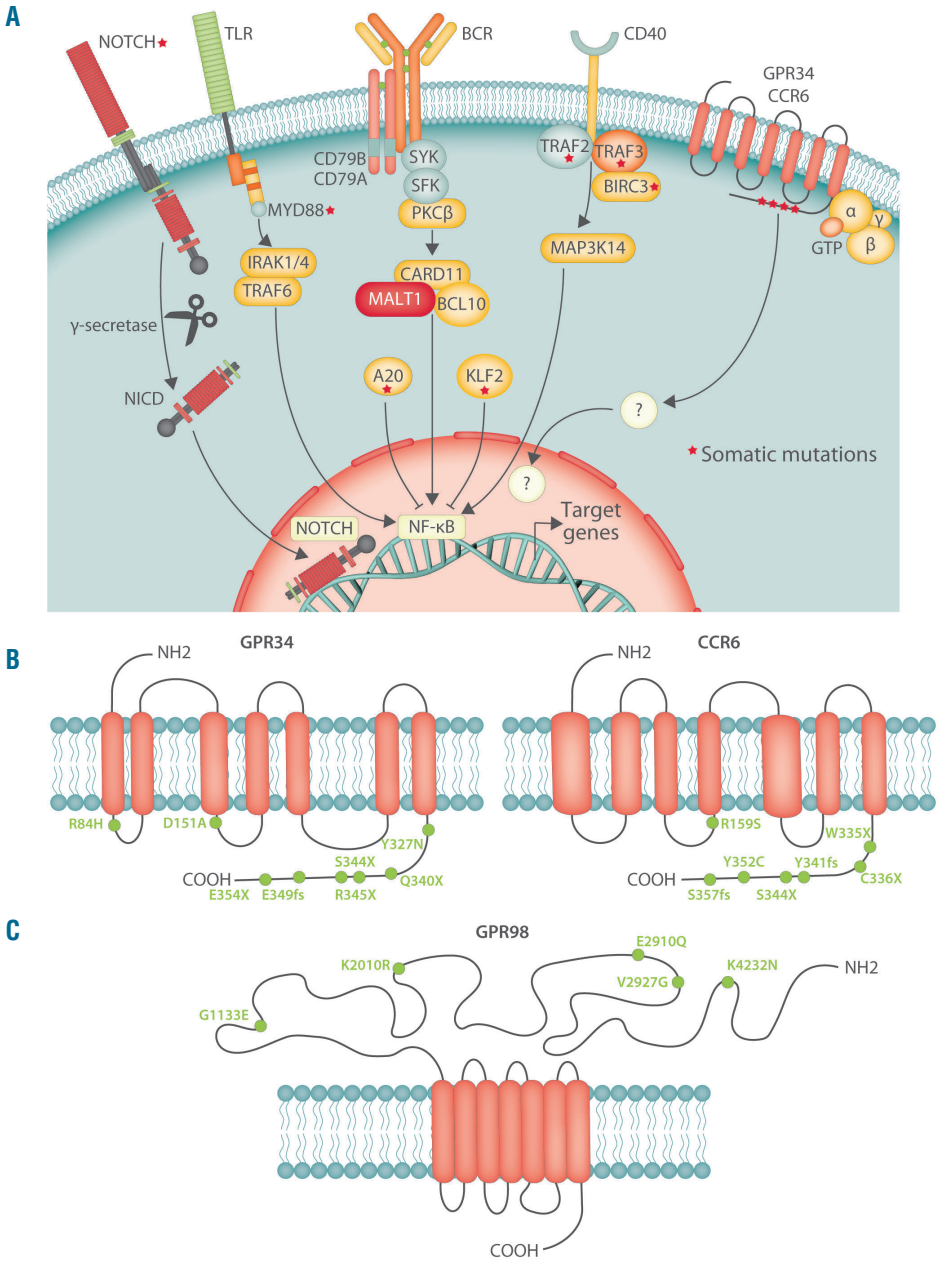

Figure 1. G-protein coupled receptor (GPCR) mutations co-operate with constitutively active NF-κB and NOTCH signaling pathways in mucosa-associated lymphoid tissue (MALT) lymphoma. (A) Representation of major signaling pathways affected by somatic genetic changes in patients with MALT lymphoma. (B) Schematic representation of GPR34 and CCR6 C-terminal mutant isoforms in MALT lymphoma (according to Moody *et al.*[11]). (C) GPR98 mutations in the extracellular domain in nodal MZL (as reported by Spina *et al.*[27]).

with GPR34 translocations or mutations were observed in the setting of autoimmune disorders,[11,17] one can hypothesize that increased amounts of lysophosphatidylserine generated in salivary gland and thyroid tissues affected by chronic inflammation could have stimulated GPR34 in surrounding B lymphocytes to progressively induce malignant transformation through the acquired GPR34 genetic lesions.

Chemokine receptor 6 (CCR6) is a chemokine receptor expressed on a variety of immune cells with a well-established role as a modulator of inflammation.[19] Most CCR6 mutations reported by Moody *et al.* are also clustered within the C-terminal cytoplasmic tail, potentially resulting in constitutive receptor triggering (Figure 1C). However, despite sharing the mutation pattern with GPR34, CCR6 is stimulated by a different ligand, chemokine CCL20, since the CCR6/CCL20 axis is involved in the function of several cell types, including memory B lymphocytes, helper and regulatory T cells, and dendritic cells.[20] Of note, a number of CCR6 missense genetic variants within the C-terminal domain have been associated with the occurrence of autoimmune disorders (Crohn disease and rheumatoid arthritis), and functional assays have demonstrated that these polymorphisms conferred decreased basal and/or ligand-induced CCR6 signaling.[21] In several other experimental models, CCL20 and CCR6 interaction promoted intestinal carcinogenesis driven by macrophage recruitment into the intestine, while disruption of CCL20-CCR6 binding inhibited cutaneous T-cell lymphoma dissemination.[19] These intriguing data provide the basis on which to define the functional role of CCR6 deregulation in MALT lymphoma.

The C-terminal distribution of mutations in GPR34 and CCR6 is similar to that observed in two other oncogenic GPCRs: C-X-C chemokine receptor type 4 (CXCR4) and C-C chemokine receptor type 4 (CCR4).[22,23] One-third of patients with Waldenström macroglobulinemia (WM), a rare lymphoplasmacytic lymphoma characterized by the constitutive MYD88 L265P activating mutation, exhibit CXCR4 mutations, which are also typical of the WHIM syndrome, an autosomal dominant immunodeficiency characterized by chronic neutropenia, hypogammaglobulinemia, recurrent infections, and myelokathexis.[24] Functional characterization of WHIM-like mutations (i.e. S338X) in WM cells showed impaired CXCR4 receptor internalization following ligand binding, which led to enhanced AKT and ERK activation.[25] Interestingly, such mutations promoted resistance to standard-of-care ibrutinib (a Bruton tyrosine kinase inhibitor) in WM cells, suggesting that additional therapies including proteasome inhibitors or CXCR4 targeting molecules could be of clinical benefit.[22] While WHIM-like mutations have rarely been described in MZLs, increased expression of CXCR4 is frequently observed in splenic, nodal and MALT lymphomas, and a functional role of this Cxcr4 overexpression triggered by constitutive BCR signaling has been shown in experimental MZL models.[15,26] On the other hand, CCR4 gain-of-function mutations located within the C-terminal domain are common in clinically aggressive adult T-cell leukemia/lymphoma, functionally leading to impaired receptor internalization and increased cell migration toward the CCL17 and CCL22 ligands.[23] Collectively, these data reveal the presence of functionally similar gain-of-function mutations in different GPCRs that are selectively observed in distinct lymphoid malignancies.

However, a different type of GPCR mutations has been recently reported in nodal MZL, an entity closely related to MALT lymphoma. Spina *et al.* used whole-exome sequencing to identify novel mutations in G-protein coupled receptor 98 (GPR98) in 5 of 35 (14%) cases, all of which corresponded to missense changes in the large GPR98 extracellular N-terminus domain of 5800 amino acids (G1133E, K2010R, E2910Q, V2927G, K4232N).[27] Interestingly, Usher syndrome type IIC, an autosomal recessive disorder characterized by congenital hearing loss and progressive retinitis pigmentosa (OMIM 605472), is caused by similar missense mutations and gene deletions within Calx-β extracellular GPR98 domains (i.e. Q2301X, S2764P, S2832X, I2906FS, M2931Fs)[28] (Figure 1C). These data, together with the Usher syndrome phenotype developed by *Gpr98* knock-out mice, suggest a loss-of-function role for extracellular GPR98 mutations that still has to be investigated. In line with these observations, two other GPCR members, the sphingosine-1-phosphate receptor 2 (S1PR2) and the P2Y receptor family member 8 (P2RY8), are recurrently mutated in germinal center (GC) mature B-cell lymphomas, preferentially by loss-of-function changes in the transmembrane domains.[29] Further underscoring a deregulated role of GPCR signaling in GC B-cell-derived tumors, loss-of-signaling mutations disrupting the GNA13 gene (encoding the Gα13 coupled protein transmitting S1PR2/P2RY8 receptor signaling) and its effector ARHGEF1 are also frequently observed, together delineating a GPCR pathway that, when disrupted, promotes the growth and blocks the dissemination of GC B lymphocytes to induce the development of GC B-cell lymphoma.[29]

In summary, there is increasing evidence to support the implication of GPCR mutations in the pathogenesis of several lymphoid malignancies. Different clinical-pathological entities show functionally similar C-terminal domain mutations in specific GPCRs that seem to impair receptor internalization and induce constitutive receptor signaling, including CXCR4 in WM, CCR6 in T-cell leukemia/lymphoma, and GPR34 and CCR6 in MALT lymphoma. Conversely, mutations in transmembrane or extracellular domains of other GPCRs can be considered loss-of-function mutations that impair downstream receptor signaling, including GPR98 in nodal MZL, and S1PR2 and P2RY8 in GC B-cell lymphomas. Such unique genetic and territorial associations strongly suggest a role of tissue-specific extracellular cues that activate selective GPCR function and disturb cell dynamics to progressively cause genetic lesions. The functional consequences of most of these genetic changes are largely unknown, particularly in the context of the obvious synergistic co-operation of specific GPCR mutations with other selected genetic abnormalities on each particular tumor type. Among them, co-existence of CXCR4 and MYD88 mutations is currently a critical issue in the diagnosis and therapy of patients with WM, while other associations such as GPR34 and TBL1XR1 mutations in MALT lymphomas of the salivary glands remain to be characterized. Looking beyond this, the present study by Moody *et al.* provides a new insight

into the pathogenesis of MALT lymphomas by linking, once again, immune receptor signaling activation and genetic abnormalities.

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