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## LYMPHOID NEOPLASIA

Comment on Korona et al, page 2186

# Turning up the heat on salivary gland MALT lymphoma

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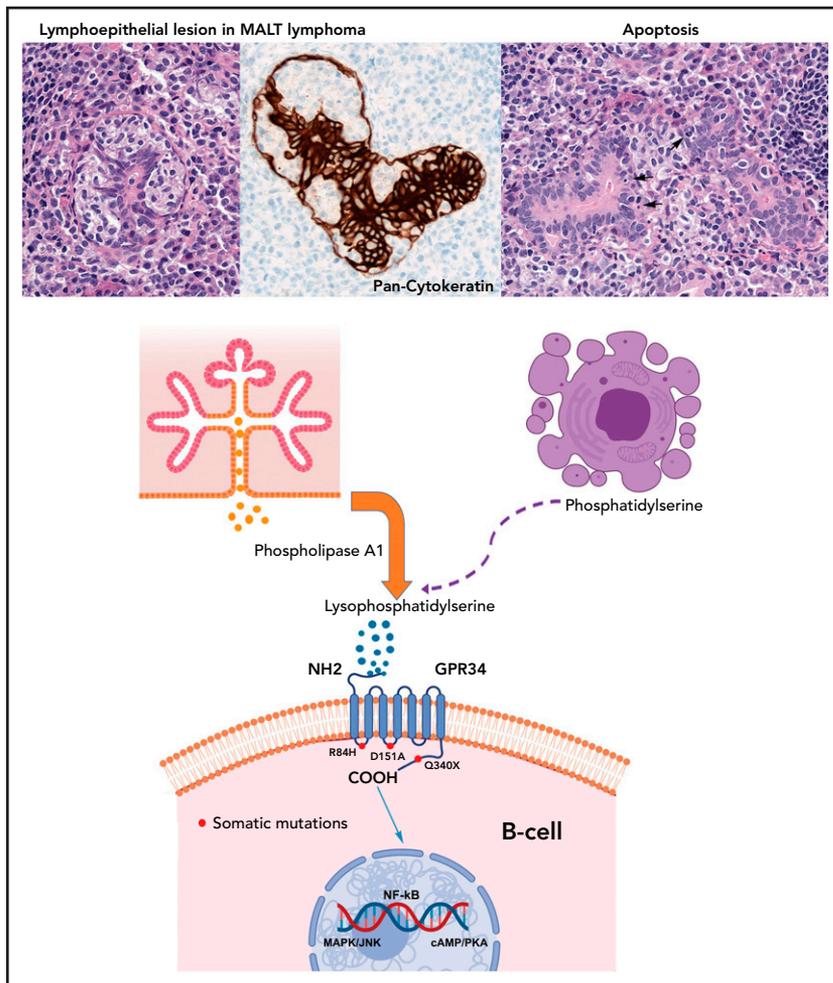
**In this issue of *Blood*, Korona and colleagues<sup>1</sup> describe a novel mechanism involving *GPR34* mutations linking the lymphoepithelial lesions of Sjögren syndrome to the development of MALT lymphoma.**

In 1971 Azzopardi and Evans described the association of myoepithelial sialadenitis, also called benign lymphoepithelial lesion (LEL) of the parotid gland, with the subsequent development of malignant lymphoma.<sup>2</sup> Today, the World Health Organization classification of lymphomas recognizes 3 types of marginal zone lymphomas (MZLs): nodal, splenic, and extranodal MZLs of the mucosa associated-lymphoid tissues (MALT). MALT lymphomas are indolent B-cell non-Hodgkin lymphomas that often arise on a background of chronic microbial infection or autoimmune disorders at diverse extranodal sites in organs that physiologically are devoid of lymphoid

tissue.<sup>3</sup> The most frequent and best characterized is MALT lymphoma of the stomach, associated with chronic *Helicobacter pylori* gastritis.<sup>4</sup> In contrast, MALT lymphomas affecting the salivary glands and the thyroid develop in the context of chronic inflammation and lymphoid hyperplasia secondary to autoimmune disorders, such as Sjögren syndrome and Hashimoto thyroiditis, respectively. Regardless of the cause of chronic inflammation, it is now recognized that this constant antigenic stimulation induces B-cell receptor (BCR) signaling and CD40 receptor activation in B cells, leading to NF- $\kappa$ B pathway activation, which triggers local proliferation of B cells that

eventually undergo malignant transformation through gaining genetic alterations.<sup>5</sup> MZLs have in common recurrent genetic aberrations that constitutively activate the NF- $\kappa$ B pathway. The most frequent oncogenic event is the inactivation by mutations or deletions of the *TNFAIP3* (*A20*) gene, a negative regulator of NF- $\kappa$ B signaling. The characteristic translocations t(11;18)(q21;q21), t(14;18)(q32;q21), and t(1;14)(p22;q32) that deregulate *MALT1*, *BCL10*, and *BIRC3* are exclusively found in MALT lymphomas and trigger NF- $\kappa$ B activation independent of antigen stimulation. In addition, there is a significant site dependence of the mutational profiles and translocation frequencies of MALT lymphomas, probably reflecting the impact of different etiologies and local microenvironments. Accordingly, *NOTCH2*, *PTPRD*, *TBL1XR1*, and *KLF2* mutations are more specific to the nodal and splenic MZLs, but similarly result in chronic BCR stimulation and further induce downstream NF- $\kappa$ B activation. More recently, recurrent somatic mutations in the G-protein-coupled receptor 34 (*GPR34*), not previously described in human neoplasias, were identified in 16% of MALT lymphomas of the salivary gland providing further evidence for the cooperation between immune receptor signaling and mutations in MALT lymphoma pathogenesis.<sup>6</sup>

*GPR34* is a member of class A G-protein-coupled receptor (GPCR) superfamily that, through G-protein activation, transduces extracellular stimuli into intracellular signals. G-protein activation promotes the intracellular changes that lead to biological responses.<sup>7</sup> Upon ligand binding, GPCRs undergo conformational changes to bind and activate heterotrimeric G-proteins at the cell membrane that in turn regulate downstream signaling targets. These receptors are closely regulated by phosphorylation in a motif localized in the C-terminal intracytoplasmic tail in most GPCRs that are responsible for interactions with  $\beta$ -arrestin. The binding of GPCR to  $\beta$ -arrestin triggers the receptor internalization and dampening of intracellular signaling, a process designated desensitization.<sup>7</sup> Interestingly, most *GPR34* mutations in salivary gland MALT lymphomas are nonsense or frameshift indels predicted to impair or delete the phosphorylation sites in the C-terminal



Paracrine GPR34 activation in salivary gland MALT lymphoma. Salivary gland MALT lymphoma (top) with typical lymphoepithelial lesion (left) highlighted by pan-cytokeratin staining (center) and increased apoptotic bodies (right, arrows). Phospholipase A1 is normally secreted by salivary gland epithelia (center left) and catalyzes generation of lysophosphatidylserine from phosphatidylserine exposed on apoptotic cells (center right). Lysophosphatidylserine binds to GPR34 and activates downstream signaling through the NF- $\kappa$ B, MAPK/JNK and cAMP/PKA pathways. The paracrine stimulation is independent of GPR34 mutational status. Red dots mark the location of the GPR34 mutations investigated by Korona et al, with the Q340X mutation in the C-terminal tail showing the most pronounced effects followed by the D151A mutation. The R84H mutation showed an effect similar to that of the GPR34 wild-type.

region and therefore deregulate desensitization of the receptor.<sup>6</sup>

In the current study Korona et al, using GPR34 expression constructs, elegantly demonstrated that GPR34 mutations observed in salivary gland MALT lymphomas, especially the Q340X truncation in the C-terminal region, confer increased resistance to apoptosis and substantially increased transformation potential. The resulting mutant also has a significantly delayed internalization after ligand stimulation and therefore remains constitutively activated and is capable of activating diverse signaling pathways critical for cellular function, such as NF- $\kappa$ B and AP1 (MAPK/JNK). Most importantly, Korona et al discovered a potential

mechanism of paracrine stimulation of malignant B-cells via GPR34 through its ligand lysophosphatidylserine generated by the unique microenvironment of LELs, independent of GPR34 mutation status. Indeed, mutated or overexpressed GPR34 is more sensitive to ligand stimulation and enhances its activation. An interesting aspect of the study was to demonstrate that phospholipase A1 (PLA1), expressed by ductal epithelium of normal salivary glands as part of its exocrine secretion, catalyzes the generation of lysophosphatidylserine from phosphatidylserine exposed on apoptotic cells present in LELs. Furthermore, the presence of local PLA1 may explain the organ tropism of GPR34 activation and mutations. The model suggests that

LELs forming in response to chronic inflammation undergoes continuous epithelial cell regeneration and apoptosis with increased ligand production perpetuating the stimulation of GPR34 in B-cells that eventually will undergo malignant transformation and acquire genetic aberrations (see figure). This important observation demonstrates that organ- and disease-specific chronic inflammatory stimuli and acquired genetic alterations play key roles in MALT lymphoma pathogenesis by dysregulating similar molecular mechanisms. Furthermore, this work is a nice example of how the investigation of the functional consequences of a mutation can lead to insights into more general pathogenetic mechanisms, in this case the generation of a paracrine stimulus for tumor growth through interaction with a specific inflammatory microenvironment.

Besides GPR34 mutations, deregulation of GPR34 has been reported in salivary gland MALT lymphomas as a consequence of a rare recurrent chromosomal translocation t(X;14)(p11;q32) that juxtaposes the GPR34 gene to the enhancers of the immunoglobulin heavy chain gene.<sup>8,9</sup> Nevertheless, elevated GPR34 mRNA expression was detected in most MALT lymphomas analyzed, regardless of the presence of the translocation indicating a functional role of this GPCR receptor in lymphomagenesis.<sup>9</sup> The role of GPR34 activation in MALT lymphomas in other organs, as well as in B-cell lymphomas in general, should be investigated to see whether receptor activation through similar mechanisms plays a role in their pathogenesis. Importantly, GPCR pharmacological targeting has shown the potential of modulating these receptors for the development of novel anticancer therapeutics<sup>10</sup> that open new treatment avenues for patients with Sjögren syndrome and salivary gland MALT lymphomas.

*Conflict-of-interest disclosure:* The authors declare no competing financial interests. ■

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## LYMPHOID NEOPLASIA

Comment on Chen et al, page 2198

# Plasticity and immune evasion in childhood ALL

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**In this issue of *Blood*, Chen et al use multiomic single-cell analysis to characterize *KMT2A*-rearranged (*KMT2A-r*) acute lymphoblastic leukemia (ALL) and show that plasticity, steroid resistance, and immunosuppressive stem-like cells are highest in the youngest patients.<sup>1</sup>**

Leukemia in the pediatric population differs from that in the adult population in both incidence and prognosis. Even within the pediatric population, large differences in both factors have been identified between infants (<1 year) and older children (≥1 year). *KMT2A-r* leukemias make up ~70% of cases of infant acute leukemias but only 10% of cases in older children.<sup>2</sup> Event-free survival is >90% for older children with ALL but 36% in infants with *KMT2A-r* ALL and <20% for patients younger than 3 months.<sup>3,4</sup> Despite the evaluation of new treatment strategies to improve prognosis, including chemotherapeutics and targeted therapies, the outcome remains poor for infants with ALL. There is a critical need for further research regarding factors underlying age-related differences in *KMT2A-r* ALL that may guide improved treatments for the youngest patients.

In this study, Chen et al apply multiomic profiling to gather cell-level information on gene expression, chromatin

accessibility, DNA methylation, and transcript fusions in patients with *KMT2A-r* ALL. Parallel measurements of these features allow researchers to form systems-level visions of how each cell works individually and with its neighbors, progenitors, and progeny. The authors use these data to uncover four key factors that are correlated with patient age at diagnosis and may determine outcome (see figure).

To investigate if tumor cells resemble heterogeneous developmental states, Chen et al performed single-cell RNA-sequencing (scRNA-seq) paired with single-cell chromatin accessibility assays (scATAC-seq) on both healthy control bone marrow (n = 5) and bone marrow from patients with *KMT2A-r* ALL (n = 25). Using bioinformatics methods to generate a developmental trajectory for these data, the authors found that *KMT2A-r* ALL leukemia cells arrest at a broad range of B-cell developmental stages, which is in line with previous studies that indicate differentiation arrest at distinct stages in B-ALL subtypes.<sup>5,6</sup> They found

that blasts from young infants (<6 months) show a significantly wider range of stages within B-cell development relative to older children, including an immature population with stem-like transcription. Further, the authors found aberrant coexpression of genes from B-cell and myeloid lineages in younger infants. Together, these findings indicate that young infants with ALL exhibit more cell state plasticity compared with older children. These features have been linked to chemotherapy resistance and poor outcomes in other hematologic malignancies.<sup>5-7</sup>

Next, the authors aimed to identify the molecular regulators and functional consequences of this increased heterogeneity among younger patients. They found that steroid response genes, such as *NR3C1* and *KLF9* transcription factors, have lower expression, lower chromatin accessibility, and higher promoter DNA methylation in younger infants. Single-cell measurements were essential for discovering these regulators, which were not evident from bulk sequencing, because the downregulated gene signatures were specifically identified in the most immature cell populations. The authors suggested that an earlier cell of origin may cause lowered steroid responsiveness and could lead to a more resistant disease. Indeed, young infants with *KMT2A-r* ALL have more steroid resistance than older children, leading to poorer outcomes.<sup>8</sup>

The phenomenon of lineage switching from a lymphoid to a myeloid fate in *KMT2A-r* ALL has been tied to poor outcomes and predominantly occurs in the younger patient population.<sup>9,10</sup> The authors provided evidence of lymphoid-to-myeloid lineage switch priming at the time of diagnosis in younger infants. This finding supports the model that *KMT2A-r* ALL originates from an early, uncommitted precursor, with a subset of patients demonstrating myeloid potential. In this study, two patients provided critical evidence that these myeloid-biased subpopulations come to the forefront under pressure by B-cell-directed immunotherapy (CART-19 or -22). Samples taken at B-ALL diagnosis (before lineage switching) showed signatures of myeloid priming, with scATAC-seq having the greatest power to detect this potential.

Finally, Chen et al gained new mechanistic insights into immune evasion, a central component of cancer development.