Kumamoto University

Mini Symposium on
Normal Hematopoiesis and Transformation:
Early Career Researchers Seminar

Date & Time : 12:30-16:45 (JST), 15th February 2023
Venue : Onsite and Online (IRCMS Lounge, Kumamoto University and Zoom)

Organizers: Goro Sashida (IRCMS, Kumamoto University)

JSPS Core-to-Core Program A. Advanced Research Networks
Integrative approach for normal and leukemic stem cells

Timetable

Opening Remarks	Hitoshi Takizawa (IRCMS, Kumamoto University)	12:30-12:35
Keynote Ⅰ (12:35-13:05)		
Masayuki Yamashita The Institute of Medical Science, The University of Tokyo	Decoding Cell Death Programs in Hematopoietic Stem Cells	12:35-13:05
Early Career Researchers Session I : Cancer (13:05-14:05)		
Mayu Kawai IRCMS, Kumamoto University	Enhanced active sub-TAD formation induced by CBFβ-SMMHC-mediated transcriptional machinery	13:05-13:17
Jie Bai IRCMS, Kumamoto University	Understanding of Trisomy 8 hematopoietic stem cell regulation and transformation by using a new Trisomy 8 model	13:17-13:29
Mayumi Hirayama IRCMS, Kumamoto University	DDX41 is required for coordinating RNA splicing and transcriptional elongation.	13:29-13: 41
Mariko Morii IRCMS, Kumamoto University	TIF1β remodels chromatin and activates the oncogenic transcriptional program in BCR-ABL leukemic stem cells	13:41-13:53
Atsuko Yonemura IRCMS, Kumamoto University	Single-cell proteomic analysis of malignant ascites in gastric cancer	13:53-14:05
Break (14:05-14:20)		
Keynote Ⅱ (14:20-14:50)		
Daichi Inoue Foundation for Biomedical Research and Innovation at Kobe (FBRI)	Altered post-transcriptional regulation in malignant hematopoiesis	14:20-14:50
Early Career Researchers Session II : Normal Hematopoiesis (14:50-15:50)		
Kenta Kikuchi IRCMS, Kumamoto University	Epigenetic regulation of dendritic cell differentiation and activation	14:50-15:02
Kiyoka Saito IRCMS, Kumamoto University	Lipoprotein metabolism mediates hematopoietic stem cell responses under acute anemic conditions	15:02-15:14
Alban Johansson IRCMS, Kumamoto University	Functional HSCs preferentially migrate to spleen during hematopoietic regeneration	15:14-15:26
Yuxin Wang IRCMS, Kumamoto University	*Akkermansia Muciniphila* induces delayed extramedullary hematopoiesis via innate immune signals	14:26-15:38
Pui Yu Ho IRCMS, Kumamoto University	Human MSC-derived bone organ improves self-renewal of transplanted human HSCs	15:38-15:50
Break (15:50-16:05)		
Keynote Ⅲ (16:05- 16:35)		
Ai Kotani The institute of medical sciences, Tokai University	Lesson from Epstein Barr virus related Hematological Malignancy	16:05-16:35
Awards ceremony and closing remarks	Goro Sashida (IRCMS, Kumamoto University)	16:35-16:45

Masayuki Yamashita
The Institute of Medical Science,
The University of Tokyo

Decoding Cell Death Programs in Hematopoietic Stem Cells

Although recent studies have unraveled the existence of abnormal hematopoietic stem cell (HSC) clones that expand with age and cause clonal hematopoiesis as well as leukemia relapse, much remains to be done to understand how such abnormal HSCs can be targeted while normal HSCs be spared. Programmed cell death is an evolutionarily conserved, ubiquitous system in multicellular organisms that eliminates rogue cells. We and others have previously demonstrated that HSCs equip multiple pathways of program cell death (e.g., apoptosis and necroptosis) that are normally inhibited by layers of HSC-specific mechanisms but can be unleashed upon stress (Yamashita et al. Cell Stem Cell 2015; Yamashita and Passegué. Cell Stem Cell 2019). We are currently striving to further "decode" HSC-specific cell death programs to find out HSC vulnerabilities that can be targeted to prevent or cure age-related or malignant blood disorders. Indeed, by utilizing various mouse models, we have identified two examples of such vulnerabilities: (1) predisposition to necroptosis pathway activation that underlies age-related HSC changes, and (2) high susceptibility of antigen-expressing HSC clones to elimination by antigen-specific cytotoxic T cells. In this presentation, I will showcase our recent data on these vulnerabilities and discuss possible strategies to selectively eliminate abnormal HSCs.

Mayu Kawai
IRCMS, Kumamoto University

Enhanced active sub-TAD formation induced by CBFβ-SMMHC-mediated transcriptional machinery

CBFβ-SMMHC is a fusion oncoprotein produced by *CBFB-MYH11* in the chromosome 16 inversion [inv(16)] de novo acute myeloid leukemia. CBFβ-SMMHC is comprised of non-DNA-binding CBFb which forms a heterodimeric transcription complex with RUNX1. We observed colocalization of CBFβ-SMMHC and RUNX1 near many activated gene loci in the leukemia-initiating cells of the *Cbfb-MYH11* knock-in mice. Furthermore, *Runx1* knockout abrogated leukemia development in the *Cbfb-MYH11* knock-in mice. These findings let us hypothesize that the transition from normal hematopoiesis to leukemic state is accompanied by global relocalization of RUNX1 genomic binding sites via interaction with CBFβ-SMMHC, leading to reorganization of promoter-enhancer interactions and thereby aberrant gene expression toward leukemogenesis.

To test this hypothesis, we collected Lineage$^-$SCA-1$^-$KIT$^+$ (LK) cells from the *Cbfb-MYH11* knock-in leukemic mice and compared the chromatin interactions to those in the control LK cells with Hi-Transposase-mediated analysis of chromatin looping (Hi-TrAC). Hi-TrAC detected 2376 control-specific and 4464 leukemia-specific chromatin loops. RUNX1 and CBFb-SMMHC often colocalized at these leukemia-specific chromatin loop anchors. Interestingly, the nested sub-TADs were prominent in the leukemic LK cells, and we detected 382 leukemia-specific active sub-TADs where the myeloid cell regulation genes were enriched.

Our data suggest that CBFb-SMMHC cooperates with RUNX1 to rewire chromatin loops for leukemia development. These observations will provide a new path forward for developing a targeting therapy in order not to rely on the current high-dose chemotherapies.

Jie Bai
IRCMS, Kumamoto University

Understanding of Trisomy 8 hematopoietic stem cell regulation and transformation by using a new Trisomy 8 model

Myelodysplastic syndrome (MDS) is a blood cancer with a poor prognosis that often occurs in elderly people. MDS is originated from hematopoietic stem cells (HSCs) and shows impaired differentiation, bone marrow dysfunction, and predisposition to acute myelogenous leukemia (AML). It has long been known that numerical chromosomal abnormalities are crucially involved in the onset and progression of MDS and AML. Numerical chromosomal abnormalities such as Trisomy 8 (+8) is an important criterion for diagnosis for MDS, but also is closely associated with clinical outcome of patients with MDS. However, due to the reproduction of trisomy in an *in vivo* setting is technically difficult, examination of transcriptional changes in genes in human MDS cells failed to determine critical genes on the chromosome 8. As a result, the pathological mechanism of +8 MDS is still unclear at levels of gene, chromosome, and cell in patients.

In order to understand the pathogenesis of +8 MDS, we have established a new Trisomy 8 model by introducing a human chromosome 8 vector in murine ES cells. We succussed to generate Trisomy 8 chimera mice from Trisomy 8 ES cells and confirmed the emergence of hematopoiesis in the fetal livers. We transplanted Trisomy 8 fetal liver cells into lethally irradiated wild-type recipient mice. Trisomy 8 mice showed myeloid-biased hematopoiesis and reduced competitive repopulating capacity of +8 HSCs, but did not develop MDS in the primary transplantation. To understand molecular mechanism of Trisomy 8-induced impaired hematopoiesis, we performed omics analyses of +8 stem and progenitor cells. Trisomy 8 HSCs showed differentially expressed genes on non-trisomic chromosomes showing increased expression of genes in type I and type II interferon responses and target genes of Polycomb Repressive Complex 2 (PRC2) and differentiation regulators such as RUNX1 and PU.1. Because human Trisomy 8 MDS cells frequently harbor loss of function mutations of the *RUNX1* gene, we attempted to determine whether *Runx1* deletion and/or *RUNX1*-mutant transduction cancel the impaired self-renewal capacity of +8 HSC and lead to developing MDS in mice. We found that *Runx1* deletion partially canceled the impaired hematopoiesis of Trisomy 8 in an *in vivo* condition, compared to *Runx1*-deleted wild-type control cells. Although we did not find a significant change in median survival between compound mice and *RUNX1S291fs* single mutant mice, transduction of *RUNX1S291fs* mutant was able to transform +8 HSC in mice, indicating that *RUNX1* mutations are seemed to be selected during the development of +8 MDS in human.

In this study, we demonstrated molecular mechanisms of impaired hematopoiesis of Trisomy 8 HSC showing alterations in epigenome and transcriptome. Trisomy 8 HSC reduced the self-renewal capacity accompanied with inappropriate activation of canonical target genes of the *RUNX1* gene, of which deletion or loss of function mutations were required for the development of +8 MDS.

Mayumi Hirayama
IRCMS, Kumamoto University

DDX41 is required for coordinating RNA splicing and transcriptional elongation

DEAD-box helicase 41 (DDX41) is one of the highly conserved DEAD-box type RNA helicases and plays multiple roles on RNA processing. The mutation of *DDX41* gene is found in 2-4% of myelodysplastic syndromes (MDS) and acute myelogenous leukemia (AML) patients and is also involved in hemocytopenia. However, the pathogenesis is not elucidated. Here, we investigated the underlying mechanism by which DDX41 dysfunction causes inefficient hematopoiesis. We found that DDX41 coordinates RNA splicing and transcriptional elongation by binding 5’ splice site. Loss DDX41 function impairs efficient RNA splicing, resulting in DNA replication stress with the accumulation of R-loop. Although the degree of DNA replication stress in S-phase is small, cells undergo mitosis with remained DNA damage, which leads to impaired cell proliferation and genomic instability. Our data suggest that these processes may be responsible for disease phenotypes associated with *DDX41* mutation.

Mariko Morii
IRCMS, Kumamoto University

TIF1β remodels chromatin and activates the oncogenic transcriptional program in BCR-ABL leukemic stem cells

Chronic myeloid leukemia (CML), characterized by expression of p210-BCR-ABL, is a clonal myeloproliferative disease initiated by malignant transformation of hematopoietic stem cells (HSC). Survival of CML patients has been significantly improved with the tyrosine kinase inhibitors, however, blastic crisis phase (BC) of CML remains a therapeutic challenge. TIF1β/KAP1/TRIM28, a chromatin modulator, both represses and activates the transcription of genes in normal and malignant cells. An analysis of published transcriptome datasets on patients with CML revealed that the expression level of TIF1β was increased in patients with CML-BC, suggesting that TIF1β is an oncogene. The deletion of Tif1β in a BCR-ABL conditional knock-in (KI) mouse model inhibited the progression of myeloid leukemia, but resulted in the development of B-cell leukemia and bone marrow failure disease. Therefore, survival was shorter in BCR-ABL KI mice, which developed CML-BC, indicating that Tif1β promoted BCR-ABL-induced myeloid leukemia. The transcription of genes involved in proliferation was increased, whereas chromatin accessibility and the transcription of differentiation regulators were decreased in BCR-ABL-expressing HSC. In contrast, chromatin accessibility and the transcription of target genes of the Fosl1/Fra1 transcription factor, which is critical for the proliferation of BCR-ABL-induced leukemia, were decreased in Tif1β-deficient BCR-ABL-expressing HSC. TIFβ directly bound to the promoter of the *FOSL1* gene in BCR-ABL-expressing cells, indicating that Tif1β remodeled chromatin and activated the transcription of genes, including Fosl1 target genes, in HSCs to drive development of leukemia. BCR-ABL KI mice did not respond to an *in vivo* treatment with dasatinib, while the deletion of Tif1β sensitized BCR-ABL-induced leukemia to dasatinib. We herein elucidated the mechanism by which the Tif1β protein regulates chromatin dynamics and the transcription of target genes in leukemic stem cells and provide it as a new therapeutic rationale for advanced leukemia.

Atsuko Yonemura
IRCMS, Kumamoto University

Single-cell proteomic analysis of malignant ascites in gastric cancer

Malignant ascites accompanied by peritoneal dissemination contains various factors and cell populations as well as cancer cells; however, how the tumor microenvironment is shaped in ascites remains unclear. Single-cell proteomic profiling and a comprehensive proteomic analysis were conducted to comprehensively characterize malignant ascites. Here, we found defects in immune effectors along with immunosuppressive cell accumulation in ascites of gastric cancer (GC) patients and identified five distinct subpopulations of CD45(-)/EpCAM(-) cells. Mesothelial cells with a mesothelial-mesenchymal transition (MMT) phenotype in CD45(-)/EpCAM(-) cells are the predominant source of chemokines involved in polymorphonuclear myeloid-derived suppressor cell (PMN-MDSC) recruitment. Moreover, MMT-induced mesothelial cells strongly express extracellular matrix (ECM)-related genes, including tenascin-C (TNC), enhancing metastatic colonization. These findings highlight the definite roles of the mesenchymal cell population in the development of a protumorigenic microenvironment to promote peritoneal dissemination.

Daichi Inoue
Foundation for Biomedical Research and Innovation at Kobe (FBRI)

Altered Post-transcriptional regulation in malignant hematopoiesis

RNA splicing factors (SFs) are among the most frequently mutated class of genes in myeloid neoplasms, particularly myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). These mutations are concentrated in four genes - SF3B1, SRSF2, U2AF1, and ZRSR2 - resulting in the aberrant splicing of pre-mRNAs. They typically manifest as heterozygous point mutations at specific residues in SF3B1, SRSF2, and U2AF1 across various forms of cancer, conferring a change-of-function; mutations in ZRSR2, located on chromosome X, result in a loss of function. Notably, ZRSR2 is unique in that it is involved in the removal of a class of evolutionarily conserved introns known as "U12" or "minor" introns. However, the functional connections between aberrant splicing events and cancer remain not fully understood. Recently, we integrated pan-cancer RNA sequencing data of SF3B1- or ZRSR2-mutated pan-cancer patients to identify mutations in SF3B1- or ZRSR2-dependent aberrant splicing events using a positive enrichment CRISPR screen to prioritize splicing alterations causing NMD that functionally promote tumorigenesis. This effort identified that diverse, recurrent SF3B1 and ZRSR2 mutations converge on the repression of BRD9 via aberrant splicing, a core component of the non-canonical BAF (ncBAF) complex, and the NMD of LZTR1, a negative regulator of Ras-related GTPases, respectively. Phenotypically, BRD9 loss remarkably promoted the development and maintenance of melanoma in a bromodomain-dependent manner. Notably, BRD9 loss induced MDS but inhibited AML development in vivo by impairing the stemness and altering lineage commitment of hematopoietic stem cells (HSCs) with myeloid skewing. Integrated scRNA/ATAC/ChIP-seq and HiC analysis of HSCs revealed that CTCF motifs exhibited striking co-localization with BRD9/ncBAF and that CTCF chromatin recruitment is augmented by BRD9 loss, leading to increased chromatin looping and expression of myeloid-related genes within intact topologically associating domains. Simultaneously, we demonstrated that the impaired minor intron excision via ZRSR2 loss enhances the self-renewal of HSCs, in stark contrast to the major "U2" type SF mutations. LZTR1 minor intron retention was also discovered in the RASopathy Noonan syndrome and diverse solid tumors due to U12 intronic mutations disrupting splicing. Indeed, loss of LZTR1, or leukemia-associated mutants in the LZTR1 substrate and RAS GTPase RIT1, which escape degradation, drive HSC expansion and leukemia in vivo. While RIT1 stabilization was sufficient to drive hematopoietic transformation, transformation mediated by LZTR1 loss required MRAS. RAS targeting bioPROTACs or reduction of GTP-loaded RAS overcomes LZTR1 loss-mediated resistance to FLT3 inhibitors. These data uncover previously unrecognized mechanisms, particularly the novel regulatory system of BRD9 and RAS pathways, for cancer development caused by mutations in SFs and have the potential to potentiate novel therapeutic strategies.

Kenta Kikuchi
IRCMS, Kumamoto University

Epigenetic regulation of dendritic cell differentiation and activation

Classical dendritic cells (cDCs) are essential for immune responses and differentiate from hematopoietic stem cells via intermediate progenitors, such as monocyte-DC progenitors (MDPs). During infection, cDCs are activated and rapidly express host defense-related genes, such as those encoding cytokines and chemokines. Recently, 3D chromatin structures have been implicated in gene regulation; however, it remains unclear how the chromatin structure of defense-related genes is reorganized during cDC differentiation and activation. In this study, we performed Hi-C on DC progenitors and cDCs to clarify chromatin structure changes at host defense-related gene loci. At the resolution achieved in this study, nuclear compartments and topologically associating domains (TADs) could be observed using our Hi-C data. We found that genomic regions containing infection inducible gene loci belonged to the active nuclear compartment throughout cDC differentiation. These genomic regions began to show increased H3K27ac levels from the MDP stage. Interestingly, intra-TAD interactions and TAD loop formation at these genomic regions were already established in the uninfected DCs and were not reinforced by infection. These results indicate that the formation of higher-order chromatin structures prior to infection contributes to the rapid responses to pathogens.

Kiyoka Saito
IRCMS, Kumamoto University

Lipoprotein metabolism mediates hematopoietic stem cell responses under acute anemic conditions

While majority of adult hematopoietic stem cells (HSCs) are maintained in a dormant state under steady-state conditions, HSCs proliferate and change their lineage output under various stress conditions such as myeloablation and infection. However, it is unclear whether and how HSCs respond to anemic conditions. We found that HSCs rapidly expand and differentiate more towards erythroid cells both in vitro and in vivo, under acute anemic conditions induced by phenylhydrazine (PHZ) treatment and phlebotomy. Gene expression profiling revealed that expression of lipid metabolism-related genes particularly very low density lipoprotein receptor (*Vldlr*) significantly elevate in HSCs of anemic mice. HSCs expressing VLDLR at a higher level ($VLDLR^{high}$HSCs) showed higher erythroid differentiation potential. Concentration of a ligand of VLDLR, apolipoprotein E (ApoE), increased in both peripheral blood and bone marrow (BM) fluid upon anemia induction while erythropoietin did not increase in the BM. HSCs co-cultured with ApoE knockout (KO) mice didn't enhance erythroid output upon PHZ treatment. We also found that $VLDLR^{high}$HSCs contain more fatty acid, and ApoE treatment increased fatty acid oxidation. ATAC-seq suggested $VLDLR^{high}$HSCs are similar to *Erg* KO HSCs that have lower megakaryopoiesis potential while $VLDLR^{low}$HSCs are similar to platelet-biased vWF^{+}HSCs. These findings suggest that ApoE regulate acute response of HSCs upon anemia induction through metabolic and transcriptomic alterations.

Alban Johansson
IRCMS, Kumamoto University

Functional HSCs preferentially migrate to spleen during hematopoietic regeneration

Extramedullary hematopoiesis (EMH) is a phenomenon that occurs under pathological conditions, e.g., during microbial infections or in certain blood disorders such as lymphomas or leukemias (Cenariu et al., 2021). During EMH, hematopoietic stem and progenitor cells (HSPCs) are produced at an increased rate in facultative niches such as liver and spleen which in steady-state conditions only contribute to a minor extent of all hematopoietic activity. However, the biological significance of extramedullary hematopoiesis remains unclear, especially during stress hematopoiesis. During regeneration of the hematopoietic system, induced by 5-FU treatment, we observed a peak of HSC expansion in spleen on Day 14 post 5-FU. Interestingly, absolute numbers of HSCs in spleen continued to increase even after reduction of divisional activity, suggesting that not only self-renewing divisions but also migration contributes to an increase in splenic HSC number after 5-FU administration. Utilizing transgenic Kaede mice (photoconversion system by UV irradiation), we directly confirmed endogenous HSCs migration from bone marrow to spleen, which in previous studies was only indirectly shown. Furthermore, steady-state spleen-derived purified hematopoietic stem cells (HSCs) showed drastically lower engraftment capacity compared to bone marrow (BM)-derived HSCs, while splenic HSCs at Day 8 post 5-FU showed equivalently high repopulation potential to BM HSCs in steady state conditions. In contrast, bone marrow HSCs at Day 8 post 5-FU injection had decreased repopulation potential compared to steady state conditions. In short, this work supports the notion that functional bone marrow resident HSCs home to spleen after myeloablation. We are currently studying potential triggers for the initiation of mobilization. From our result, we now hypothesize that HSCs temporarily escape from inappropriate or harmful niche environments within damaged BM after 5-FU administration to maintain stem-ness.

Yuxin Wang
IRCMS, Kumamoto University

Akkermansia Muciniphila induces delayed extramedullary hematopoie sis via innate immune signals

Abstract: Hematopoietic stem and progenitor cells (HSPCs) reside in bone marrow (BM) and can be mobilized from BM to the spleen under hematopoietic stress conditions such as infection, inflammation, and anemia, which is called extramedullary hematopoiesis (EMH). Recently, the microbiota has been shown to regulate the host immune system and induce mobilization of HSPCs, suggesting gut microbiota might play some roles in EMH. *Akkermansia muciniphila* (*A. m.*) is one of the mucin-degrading gut microbial bacteria in humans and rodents that involves metabolic changes and immune regulation. However, few studies have examined the effect of *A. m.* on hematopoiesis.

Here, we injected wild type mice with a single shot of *A. m.* lysate containing cell membrane fraction and induced rapid activation of BM myelopoiesis and a slow, but long-lasting hepato-splenomegaly characterized by the expansion of functional HSPCs, which we termed chronic EMH. Mechanistically, *A. m.*-induced splenic EMH was mediated entirely by MYD88/TRIF-dependent innate immune signals and partially by TLR2/4 dependent signals. IL-1a was persistently secreted by splenic dendritic cells and Ly6C$^+$ monocytes activated IL-1R-expressing splenic HSPCs. Moreover, pharmacological inhibition of IL-1R in addition to TLR2/4 genetic ablation completely canceled EMH, which indicated cooperative roles of IL-1R and TLR signals in *A. m.* induced EMH. These novel findings might be involved in the pathophysiology of *A. m.*-associated metabolic and inflammatory disorders and IL-1-related chronic hematological and immunological disorders such as stem cell aging, clonal hematopoiesis and autoimmune diseases.

Pui Yu Ho
IRCMS, Kumamoto University

Human MSC-derived bone organ improves self-renewal of transplanted human HSCs

While hematopoietic stem cells (HSCs) normally reside in bone marrow (BM), a small fraction of HSCs routinely and homeostatically migrate outside to blood stream and back inside BM by HSC mobilisation and homing, respectively. HSC homing is a critical process for HSC transplantation (HSCT), in which efficiency is often limited by inferior HSC homing conditions and engraftment rate. However, majority of current models for HSC homing study is heavily murine based. Our previous work found that humanized bone organ (hOssicle) derived from human mesenchymal stem cells (hMSCs) can sustain the quiescence of transplanted human HSCs (hHSCs) *in vivo*, compared to the mouse bone marrow (Fritsch K et al, Exp Hematol 2018).

Taking advantage of humanized niche in our xenograft model, we aim to investigate how the HSC-niche interaction regulates the hHSC homing and engraftment after HSCT. We found that in mouse BM, the cord blood (CB)-derived hHSCs quickly differentiate to downstream progenitor cells within 2 weeks post-transplantation. In contrast, the hOssicle can support the expansion of hematopoietic progenitors while preserving the stem cell fitness of hHSCs by limiting their cell division frequency. The hHSCs in hOssicle also showed more suppressed apoptosis compared to their counterparts in mouse BM. By analysis of mitochondrial ROS production, we found that the oxidative stress was reduced not only in hHSCs but also the niche cells inside the hOssicle.

These data suggest that compared to the mouse BM niche, humanized microenvironment is more supportive to the transplanted hHSCs possibly by regulation of oxidative stress, which may prevent the engrafted hHSCs from ROS-induced DNA damage and thus maintain their self-renewal ability. Our niche humanization model can serve as a versatile system to better understand human HSC and BM niche biology *in vivo*.

Ai Kotani
The institute of medical sciences,
Tokai university

Lesson from Epstein Barr virus related Hematological Malignancy

Extracellular vesicles are an area that has received increasing attention in recent years, and this is especially true in the field of cancer. One reason is that cancer cells secrete larger amounts of vesicles than normal cells. Knowledge has accumulated on the mechanism and its significance. In addition, although studies have been conducted mainly on small RNAs and proteins, recently, analyses of lipids and carbohydrates, which are molecular species not attributed to genes, have begun, and their biological significance and new mechanisms of action are also being clarified. I would like to introduce them, their clinical applications, and my own research on lymphoma.

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