order to thrive. Interestingly, we revealed a dramatic increase of immature granulocytes only in bone marrow of EBV-infected mice. In addition, GM-CSF, a cytokine that is essential for differentiation of the myeloid lineage, was significantly increased in EBV-infected mice. These results were also reproduced in patients with EBV-related disorders.

This research revealed that abnormal hematopoiesis was occurred in mice following EBV infection. Considering the above results, the hematopoietic alteration might be involved in a cause of lymphoproliferative disorders in human by reduction of tumor immunity.

2-3.
Expression of intracellular cytokines in twins with intractable epilepsy associated with lissencephaly

目的：免疫学的参与在癫痫的发病机制中的作用已得到建议。我们旨在确定是否存在无症状的小儿性癫痫的免疫系统功能异常。尽管共享相同的诊断为小脑性癫痫和相同的基因突变，这些双胞胎的发展预后不同：双胞胎A有一个严重的发育延迟，而双胞胎B有一个适度的发育延迟。

方法：通过流式细胞仪，我们分析了双胞胎的外周血单核细胞的细胞内 cytokine profile and plasma cytokine levels in reference to two age-matched controls.

结果：双胞胎有较高的 interleukin-1 beta (IL-1β)-positive CD14+ monocytes than the controls. Twin A had higher percentages of IL-1 receptor antagonist (IL-1RA)-positive and tumor necrosis factor-alpha (TNF-α)-positive CD14+ monocytes than twin B and the controls. The plasma cytokine levels of IL-1β, IL-1RA, and TNF-α were lower in twin A than in twin B and the controls.

结论：虽然一份双胞胎之间 cytokine-producing cells 的比例存在差异，但尚不清楚这种差异如何影响其各自的病理生理学。进一步的研究需要更多的患者来评估少数 cytokine-producing cells 的数量差异是否可以影响难治性癫痫的发病机制。

3-I-1.
DNA-damaging drugs in combination with a macrolide antibiotic enhance cytotoxicity for non-small cell lung cancer cells

For the treatment of lung cancer, new drugs such as tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors are being developed one after another, but patients who show interstitial pneumonia or do not carry gene mutations of targets for each TKIs are not applicable for them. Therefore, conventional DNA-damaging drugs are still useful for such patients. In recent years, it has been revealed that cancer cells utilize autophagy for their own growth and that autophagy acts cytoprotectively when cancer cells are exposed to antitumor drugs. Because hydroxychloroquine (HCQ) is the only clinically available autophagy inhibitor but causes severe retinopathy and cardiomyopathy, the development of other autophagy inhibitors is desired. Our group has been reported that macrolide antibiotics have an autophagy inhibitory effect and that their combined use with TKIs or proteasome inhibitors
enhanced cytotoxicity in various cancer cells. In this study, we evaluated the effect of combination therapy with DNA-damaging drugs and a macrolide antibiotic.

We found that DNA-damaging drugs in combination with azithromycin (AZM), one of the macrolide antibiotics, enhanced cell death including apoptosis in a non-small cell lung cancer cell line, A549. This enhanced cytotoxicity by the drug combinations was significantly decreased in a p53-mutated lung cancer cell line and a p53 KO A549 cells. We also found that the combined use of a DNA-damaging drug and AZM significantly changed the morphology with an increased number of the enlarged LAMP2-positive organelles which were considered as autolysosomes and/or lysosomes. These data suggested that DNA-damaging drugs in combination with AZM strongly induced cell death in A549 cells by activating apoptosis which might be caused by the damaged lysosomes and dependent on the p53 signaling pathway.

3-1-2. Increased APOBEC3C–H Gene Expression is Associated with Improved Outcome in Breast Cancer

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Background: APOBEC3 are strong mutagenic enzymes. The association between APOBEC3B (A3B) expression and DNA mutation has been well studied. However, it is still unclear on other A3s (A3A, C-H). We investigated the clinical relevance of A3s on their mutagenic and cancer immunity angles.

Methods: 1) A3s gene expression level was examined on 55 breast cancer cell lines.

2) The association of A3s with the clinical outcome and other molecular features were investigated from TCGA-BRCA data. Patients were divided into 3 groups by the A3s gene expression level: high, intermediate and low. The clinical outcome were compared between high and low groups. Molecular features were quantified with bioinformatics workflow and examined the association with A3s expression level.

Results: 1) A3B & 3C represented 91% of A3s expression in cell lines. 2) A3C–H expression was significantly associated with improved clinical outcome (HR, 0.45–0.66). A3A and A3B expression levels were correlated with both tumor mutation burden and neoantigen load (Spearman r = 0.28–0.34), while not for A3C–H. Expression of genes related to immune function like interferon response and complement activation was enriched in high A3C–H expressors, which significance was observed in CD4 and CD8 T cells, TCR diversity and tumor immune cytolytic activity (2.3–4.0x, 2.1–5.4x, 1.3–2.1x & 3.1–7.9x, resp.).

Conclusion: Unlike A3B, A3C–H were expressed in stromal cells, not in breast cancer cells. A3C–H expression may activate immune cells. A3C–H gene expression was associated with better outcome in breast cancer patients. It is speculated that up-regulating immune function by A3C–H may explain this clinical finding.