Molecular Pathology Abstracts

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Genetics and pathogenesis of diffuse large B-cell lymphoma

Understanding the genetic basis of diffuse large B-cell lymphoma is important for understanding the pathogenesis of the disease and the molecular attributes that may influence therapeutic response. Diffuse large B-cell lymphoma (DLBCL) is divided into the subgroups of germinal center B cell like (GCB), activated B cell like (ABC), and unclassified, based on gene-expression profiling. This distinction has strong prognostic value and predicts response to standard treatment. Patients with ABC-type refractory DLBCL respond better to ibrutinib, an inhibitor of B-cell receptor-dependent NF-κB activation, when *CD79B* and *MYD88* mutations are present. The authors sought to extend these findings by studying structural genomic abnormalities and gene expression in DLBCL biopsy samples to uncover therapeutic targets based on tumor genetics. They analyzed 574 fresh-frozen DLBCL biopsy samples (51.4 percent ABC, 28.6 percent GCB, and 20 percent unclassified) using exome and transcriptome sequencing, deep amplicon resequencing of 372 genes, and DNA copy-number analysis. Using an algorithm to identify the genetic distinction among gene-expression subgroups, the authors identified four genetic subtypes of DLBCL in 46.6 percent of the cases studied: MCD, based on the co-occurrence of *MYD88*^{L265P} and *CD79B* mutations; BN2, based on *BCL6* fusions and *NOTCH2* mutations; N1, based on *NOTCH1* mutations; and EZB, based on *EZH2* mutations and *BCL2* translocations. The MCD subtype had frequent gain or amplification of *SPIB*, as well as mutations in the tumor suppressors *CDKN2A, ETV6, BTG1,* and *BTG2* and the putative tumor suppressors *TOX, SETD1B, FOXC1, TBL1XR1,* and *KLHL14*. Immune editing was prominent in MCD genomes, with 76 percent of samples harboring a mutation or deletion of *HLA-A, HLA-B,* or *HLA-C* and 30 percent of cases showing a truncating mutation of CD58. The BN2 subgroup was dominated by NOTCH pathway aberrations, such as *NOTCH2* mutation or amplification; *SPEN* mutation; *DTX1* mutation, a NOTCH target gene; and genetic alterations targeting the NF-κB pathway. *BCL6* fusions were present in 73 percent of BN2 cases. The N1 subtype was characterized by *NOTCH1* mutations and aberrations targeting transcriptional regulators of B-cell differentiation. The EZB subtype included BCL2 translocations, EZH2 mutations, and REL amplification, as well as inactivation of the tumor suppressors *TNFRSF14, CREBBP, EP300*, and *KMT2D*. The germinal-center homing pathway involving *S1PR2* and *GNA13* was disrupted in 38 percent of EZB cases. Loss-of-function lesions targeting negative regulators of B-cell receptor signaling aberrations occurred in all genetic subtypes, indicating that B-cell receptor signaling is an important aspect of DLBCL pathogenesis. In addition to the DLBCL subtypes harboring different gene-expression signatures, they responded to immunochemotherapy differently, with the BN2 and EZB subtypes demonstrating favorable outcomes and the MCD and N1 subtypes showing poorer outcomes, possibly due to genetic heterogeneity. When looking at survival outcomes within the ABC DLBCL, the MCD subgroup had survival rates that were significantly inferior to those of the BN2 subgroup. And patients with MCD or N1 had survival rates that were significantly inferior to those with ABC tumors that were not classified genetically. Within GCB DLBCL, E2B patients had survival rates that were inferior to those with other GCB tumors. MCD and BN2 tumors showed B-cell receptor-dependent NF-κB activation, suggesting that drugs targeting these pathways should be investigated for therapeutic purposes. This study extends the use of genomic profiling for the classification of DLBCL and provides insights into pathogenesis and possible therapeutic interventions.

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Somatic activating KRAS mutations in arteriovenous malformations of the brain

Arteriovenous malformations of the brain are tortuous, morphologically abnormal vascular channels between arteries and veins that lack an intervening capillary network. This allows high-pressure arterial blood from feeding arteries to shunt directly into the venous outflow system. If an arteriovenous malformation (AVM) ruptures, it can cause hemorrhage, stroke, or brain damage. The underlying cause of AVM is unknown, but rare genetic disorders, such as hereditary hemorrhagic telangiectasias and capillary malformation–arteriovenous malformation syndrome, exhibit similar lesions. To explore the hypothesis that brain AVMs may result from somatic mutations in cranial vascular cells, the authors analyzed paired tissue and blood samples from patients with sporadic unifocal AVMs by whole exome sequencing. They used fresh-frozen tissue samples from 39 patients (21 with matched blood samples) who served as the main study group and paraffin-embedded tissue samples from 33 Finnish patients who served as an independent validation group. Whole exome sequencing performed on tissue samples of AVMs of the brain from 26 patients in the main study group found activating *KRAS* mutations (c.35G>T [p.Gly12Val] and c.35G>A [p.Gly12Asp]) in 12 of the patients and none of the 17 paired blood samples. To confirm these results and detect *KRAS* variants that might have been missed due to low representation in the sample, the authors performed droplet digital polymerase chain-reaction analysis on the aforementioned 26 patients and 13 additional tissue samples, four of which had matching blood samples. The digital droplet PCR confirmed the *KRAS* mutations in the original 12 patients and revealed *KRAS* mutations in the tissue samples of 29 of the 39 patients overall but not in any of the 21 paired blood samples. Sixteen of 33 tissue samples from the Finnish group were also positive for *KRAS* variants. To identify the types of cells harboring *KRAS* mutations in brain AVMs, endothelial cells were enriched from cell cultures derived from AVM tissue using anti-CD31 magnetic beads. In AVM cultures from five of six patients previously shown to harbor a *KRAS* mutation, a large percentage of the cells from the enriched cell populations demonstrated the same *KRAS* mutation. Finally, the authors showed an associated increase in phosphorylation of ERK1/2, a downstream mediator of *KRAS*, in cultured cells that were positive for the *KRAS* mutation, whereas three control CD31+ cell cultures derived from normal brain vasculature did not show increased phosphorylation of ERK1/2. Immunohistochemical staining of tissue samples that were positive for a *KRAS* mutation showed strong staining for ERK phosphorylation in endothelial cells lining the vascular lumen and in vascular smooth-muscle cells, whereas normal brain parenchymal vessels showed little or no staining for ERK phosphorylation in endothelial cells. These findings suggest that mutant *KRAS* specifically activates the MAPK–ERK pathway in endothelial cells. The presence of *KRAS* variants in endothelial cells suggests that dysregulation of endothelial cells may be a key feature of the formation of brain AVMs and that these variants probably arise early in development. The evaluation of MEK inhibitors in the treatment of AVMs may warrant further study.

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