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ORAL ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Emergence of Highly-Plastic B Cell States Cooperates with Early Immune Microenvironment Remodeling to Drive Follicular Lymphomagenesis

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Follicular lymphoma (FL) is a prototypical example of B cell lymphoma arising from germinal center (GC) B cells. Recent single cell transcriptomic studies investigating the transcriptional heterogeneity of FL suggest that tumor B cells are functionally diverse with cells acquiring an intermediate phenotype between GC and memory (mem), in line with the premalignant FL ontogeny where BCL2 $^{+}$ memory B cells require multiple re-entries into the GC to facilitate the transformation process. Since FL cells often hijack the epigenetic machinery through mutations in histone modifying proteins - notably *KMT2D* - we questioned here how epigenetic alterations contribute to the emergence of this intratumoral heterogeneity.

We developed a genetically engineered mouse FL-like model (GEMM) carrying *Kmt2d* loss-of-function in B cells and *BCL2* overexpression recapitulating different progression stages from preneoplastic hyperplasia to FL-like tumors. We analyzed 45,541 high-quality single cell transcriptomes and immune repertoires (B cell receptor, BCR and T cell receptor, TCR) from 18 mice including immunized-control mice in the context of physiological GC reaction. We developed a computational workflow on mutant B cells combining supervised and unsupervised metaclustering approaches in order to highlight recurrent gene programs active in preneoplastic hyperplasia and the various stages leading to FL-like tumors. We used *inferCNV* to assess the association between genetics and transcriptomic heterogeneity and inferred cell-cell interactions to characterize the microenvironmental determinants associated with B cell heterogeneity throughout FL progression.

By modeling the GC cycle dynamics and building B cell signatures during a normal immune response, we found that our FL GEMM mirrored the desynchronization of the GC reaction program previously observed in human FL. Single cell expression profiles of *Kmt2d-BCL2* mutant cells also showed a reproducible increase in phenotypic heterogeneity during FL progression, manifesting as a continuum of cell states from proliferating GC-like to quiescent mem-like cell states. We defined this GC-to-Mem axis as the major source of intra-tumor transcriptional heterogeneity. Single-cell BCR sequencing revealed the emergence of highly-plastic B cell states during FL evolution, co-existing within individual mice and being largely independent of subclonal genetic variation. To further elucidate the emergence and maintenance of these cell state transitions, we explored the phenotypic and transcriptional changes within the immune microenvironment along disease progression. Significant compositional changes were found, including the expansion of T follicular helper cells and T regulatory T cells together with the loss of naive CD4, CD8 T, and NK cell populations, preceding malignant transformation and creating a (pre)tumoral supportive niche persisting in advanced tumors. Prior remodeling of a premalignant supportive immune niche may be crucial in establishing malignant cell heterogeneity. Ongoing efforts aim to impair tumor-immune interactions driving tumor B cell dynamics and survival.

In conclusion, our results provide a high-resolution view of events spanning FL progression and indicate a major role of early TME remodeling in establishing a suitable niche for progression. Early intervention aimed to target the B cell-tumor

microenvironment interactions driving intra-tumoral heterogeneity may represent a promising therapeutic avenue against early disease and prevention of FL recurrence.

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