（様式 8）Form 8

学位論文内容の要旨
（Summary of Dissertation）

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Research on tumor microenvironment of brain metastases lung adenocarcinoma
(脳転移性肺腺癌の腫瘍微小環境に関する研究)

【Background and Objectives】

Lung cancer is a leading cause of cancer mortality worldwide, with lung adenocarcinoma (LUAD) being a predominant subtype. A frequent and devastating complication is brain metastasis (BM), which occurs in up to 55% of patients, drastically shortening survival. Patients with EGFR-mutated LUAD face an even higher risk of BM. Research progress has been hindered by difficulties in obtaining patient-derived BM cells and limitations in existing animal models used to create cell lines. Current methods, such as left ventricular injection, are reliable but costly and time-consuming. A more common approach, direct intracranial injection, is faster but typically uses a large number of cells, which is biologically inconsistent with the natural colonization process and may produce unreliable cell lines. This study aimed to develop an improved, more rapid, and biologically relevant method for establishing BM-LUAD cell lines using low-cell-number injections. The objectives were to characterize these new cell lines to understand the dynamic evolution of brain metastasis and to validate their similarity to clinical samples.

【Materials and Methods】

The study utilized the EGFR-mutated H1975 human LUAD cell line as the parental line, with all cells cultured in standard media. All animal experiments used female nude mice under approved ethical protocols. To establish the metastatic cell lines, an improved method of low-cell-number serial intracranial injection into the hippocampal region was performed. This process was repeated three times, using 500, then 4000, and finally 4000 cells, to sequentially generate the H1975-BM1, H1975-BM2, and H1975-BM3 cell lines. After each cycle, brain tumor tissue was extracted, cultured, and used for the next injection round. The metastatic potential of the final cell line was validated by injection into the left ventricle, with tumor growth monitored via an IVIS imaging system. In vivo growth characteristics were also assessed using subcutaneous xenografts. In vitro functional assays included Transwell assays for migration, adhesion assays on ultra-low attachment plates for cell clustering, and CCK-8 assays for proliferation. For molecular analysis, bulk RNA sequencing was conducted on all cell lines. A novel bioinformatic workflow was created to assess clinical relevance by analyzing public scRNA-seq data (GSE131907) and using the CIBERSORT algorithm to compare the transcriptomic signatures of the established cell lines with those of patient-derived tumor cell subpopulations. Finally, Weighted Gene Co-expression Network Analysis (WGCNA) was used to identify gene modules and key hub genes associated with different metastatic stages.

【Results】

1. Establishment and Validation of Aggressive BM Cell Lines

The low-cell-number serial intracranial injection method successfully generated three distinct brain metastatic cell lines: H1975-BM1, BM2, and BM3. Survival studies showed that mice injected with the later-stage BM2 and BM3 cells had significantly shorter survival times than those injected with the early-stage BM1 line, confirming

that the cell lines became progressively more aggressive. To validate true metastatic capability, cells were injected into the left ventricle. This experiment demonstrated that 50% of mice injected with BM3 cells developed brain metastases, whereas none in the parental H1975 group did, confirming that the BM3 line has a strong tropism for brain colonization.

2. Phenotypic Evolution During Brain Metastasis

In vitro assays revealed unexpected phenotypic shifts. The early-stage BM1 line showed the highest migratory capacity, while the late-stage BM3 line was the least migratory. Conversely, the BM3 line exhibited the strongest cell-cell adhesion, forming large cell clusters, a trait associated with higher metastatic potential in circulating tumor cells. In vitro proliferation was highest in the parental H1975 cells and progressively decreased in the BM lines. However, in vivo results contrasted with this; subcutaneous tumors formed by BM3 cells were significantly larger and contained more secretory fluid (cysts) than those from BM1 cells, indicating enhanced proliferation and secretory activity within a physiological microenvironment.

3. Similarity to Clinical Samples and Transcriptomic Evolution

Analysis of clinical scRNA-seq data identified six tumor cell subpopulations (Tumor C0-C5), of which C0, C1, and C3 were significantly enriched in patient BM samples. The novel deconvolution workflow revealed that the proportions of these BM-specific subpopulations (Tumor C0 and C1) steadily increased from the parental H1975 line to the advanced BM3 line. This confirmed that the cell lines progressively acquired the transcriptomic signatures of clinical brain metastases, validating their reliability.

4. WGCNA identified distinct gene co-expression modules associated with each metastatic stage.

The BM1-specific module (brown) was enriched for genes involved in Wnt signaling, stemness, and extracellular matrix (ECM) organization, aligning with its high migratory and adaptive phenotype. The BM3-specific module (greenyellow) was enriched for pathways related to chemotaxis, cell adhesion, and inflammatory responses, consistent with its phenotype of enhanced clustering and interaction with the tumor microenvironment. From this module, RAB25 was identified as a key hub gene, and its protein expression was found to be significantly upregulated in clinical BM-LUAD samples compared to primary tumors.

【Discussion】

This study introduces a valuable alternative to traditional methods for generating BM cell lines, overcoming the high cost and time of ventricular injections and the biological irrelevance of high-cell-number intracranial models. The developed low-cell-number injection method better mimics the natural metastatic process, and the innovative bioinformatic pipeline provides crucial validation of the cell lines' similarity to patient tumors. The results depict a clear evolutionary trajectory during brain colonization. The early-stage BM1 phenotype (high migration, stemness) reflects the initial adaptation and invasion phase. The late-stage BM3 phenotype (high adhesion, lower migration) is consistent with mesenchymal-to-epithelial transition (MET), where cells stabilize to form a proliferative colony, a phenomenon observed in clinical metastases. The enhanced clustering ability of BM3 cells is particularly noteworthy, as this trait is known to increase metastatic efficiency. The identification of the hub gene RAB25, previously linked to therapy resistance in lung cancer, as a key player in advanced BM provides a promising new target for investigation. While the intracranial model does not fully recapitulate the entire metastatic cascade, this work provides highly reliable tools and insights into the progression of BM-LUAD.

【Conclusion】

This study successfully developed and validated a low-cost, rapid, and more biologically relevant method to establish BM-LUAD cell lines. A novel bioinformatic pipeline confirmed that these cell lines recapitulate the transcriptomic features of clinical samples. The research revealed that tumor cells evolve during brain colonization, with early-stage cells exhibiting high stemness and migration, while late-stage cells show enhanced cell adhesion and secretory functions. Finally, RAB25 was identified as a potential key driver of BM-LUAD progression that warrants further investigation.