主論文の要旨

Combination of tumor necrosis factor-α and epidermal growth factor induces the adrenergic-to-mesenchymal transdifferentiation in SH-SY5Y neuroblastoma cells

（腫瘍壊死因子−α と上皮成長因子の組み合わせは
神経芽腫細胞 SH-SY5Y においてアドレナリン作動性から
間葉系への相互転換を誘導する）

名古屋大学大学院医学系研究科　総合医学専攻
発育・加齢医学講座　小児科学分野
（指導：髙橋 義行　教授）
黄 越

【Introduction】

Neuroblastoma, a type of cancer that is common in children, is composed of epigenetically regulated two distinct cell types: undifferentiated mesenchymal (MES) and lineage-committed adrenergic (ADRN) types. MES-type cells are more migratory, resistant to chemotherapy, and prevalent in relapse tumors. Importantly, MES- and ADRN-type cells can spontaneously transdifferentiate into one another, and the interconversion can be controlled by lineage-specific genes. Although MES- and ADRN-types are well characterized and experimentally controllable by genetic manipulations, the mechanisms of its spontaneous transdifferentiation driven by external or environmental stimuli have not yet been elucidated. In the present study, we aimed to identify the extracellular factors that can induce/promote transdifferentiation between MES- and ADRN-type cells and understand the molecular mechanisms of the epigenetically controlled intratumor heterogeneity of neuroblastoma.

【Materials and methods】

Gene set enrichment analysis (GSEA) was performed to select candidate factors using public data sets. Two clonally expanded neuroblastoma cell lines, SH-EP (MES-type) and SH-SY5Y (ADRN-type), were used. Quantitative RT-PCR, western blotting, scratch wound cell migration assay and chemoresistance assay were performed to assess the effects of candidate factors on transdifferentiation. A publicly available data was used to investigate expression of genes in human neuroblastoma.

【Results】

We performed GSEA for a gene expression matrix comparing MES- and ADRN-type cells, and selected 6 ADRN and 10 MES candidate factors (Table 1).

First, we investigated whether a combination of 6 ADRN or 10 MES candidate factors could differentially regulate MES or ADRN genes. We found that a combination of 10 MES factors clearly induced the MES gene expression profile in the ADRN-type SH-SY5Y neuroblastoma cells. The structure of SH-SY5Y cells treated with 10 MES factors resembled that of MES-type cells, showing flattened cell shape with reduced neurite structures (Figure 1A). Importantly, a combination of 10 MES factors modestly upregulated a MES marker gene YAP and strongly downregulated ADRN genes PHOX2A, PHOX2B, and GATA3 in SH-SY5Y cells, indicating an ADRN to MES transition (Figure 1B). Since a previous study has identified super-enhancer-associated lineage transcription factor (TF) networks in MES- and ADRN-types, we selected 5 MES and 5 ADRN TF genes which were differentially expressed between SH-EP and SH-SY5Y cells for qPCR experiments. Importantly, all 5 MES and 5 ADRN TF genes were up- and downregulated, respectively, in SH-SY5Y cells treated with a combination of 10 MES factors (Figure 1C).

To determine which of the 10 MES factors were essential, we examined the effect of withdrawal of individual factors from a combination of 10 MES factors on the expression of 5 MES and 5 ADRN TF genes in SH-SY5Y cells. Consistent with Figure 1C, all 5 MES or 5 ADRN TF genes were greatly up- or downregulated in SH-SY5Y cells treated with a combination of 10 MES factors, respectively (Figure 2A). Importantly, this up- or downregulation of MES or ADRN TF genes was attenuated by the removal of IFN-γ, TNF-α or EGF, prominently observed in three MES TF genes (*EGR3*, *WWTR1*, and *SOX9*) and all 5 ADRN TF genes (Figure 2A). Accordingly, withdrawal of these three factors resulted in a more obvious attenuation of all 5 MES or all 5 ADRN TF gene upregulation or downregulation (Figure 2B).

To identify the significant factors that induce the MES gene expression profile, we investigated whether treatment with a single or combination of three factors (IFN-γ, TNF-α, and EGF) could produce the effects comparable with those of a combination of 10 MES factors on the expression of MES and ADRN TF genes. Treatment with all three components or a combination of TNF-α and EGF did not completely but comparably up- or downregulated the MES or ADRN TF genes compared with treatment with a combination of all MES factors (Figure 2C).

As shown in previous studies, higher migration ability and chemoresistance are important characteristics of MES-type neuroblastoma cells. Importantly, the combination of TNF-α and EGF significantly promoted the migration ability of SH-SY5Y cells, as early as 10 h (Figure 3A) and resistance to anti-cancer drugs, etoposide and doxorubicin (Figure 3B).

Finally, we investigated whether our findings are relevant to human neuroblastoma using a cohort including 649 human neuroblastomas from R2 database. Importantly, the expression of both TNF and EGF receptors was strongly associated with MES signature in human neuroblastoma (Figure 4A), suggesting that intracellular signaling mediated by these receptors might control the interconversion between ADRN and MES types.

【Discussion】

In the current study, we performed GSEA to select 6 ADRN and 10 MES candidate factors that can possibly induce ADRN and MES expression profiles, respectively. Treatment with a combination of 10 MES factors clearly induced the MES gene expression profile in SH-SY5Y cells (ADRN type). Based on the expression of super-enhancer-associated lineage TFs, migration ability, and chemoresistance, we narrowed down and identified that the synergistic combination of TNF-α and EGF induced the MES state. Importantly, the expression of both TNF and EGF receptors was strongly associated with MES signatures in human neuroblastoma, suggesting that intracellular signaling mediated

by these receptors might control the interconversion between ADRN and MES types or the identity of MES-type neuroblastoma cells.

Interestingly, a previous study demonstrated that the acquisition of cisplatin resistance (a characteristic of MES-type cells) accompanied with increased EGFR expression in neuroblastoma cells, making them highly sensitive to EGFR-targeted therapy. EGF promotes the proliferation of neuroblastoma cells, as observed in this study. TNF-α can also increase the cell proliferation rate in two neuroblastoma cell lines (SK-N-BE and SK-N-FI), and antibodies targeting TNF or its receptors inhibited cell growth; however, we observed an opposite effect in SH-SY5Y cells, probably due to the induction of neuroblastoma cell differentiation as shown previously. In addition, ionizing radiation induces a TNF-α-NFκB positive feedback loop, which is advantageous for the survival of neuroblastoma cells. Because MES-type neuroblastoma cells are more prevalent in relapse neuroblastoma, resistance to chemotherapy or radiation might be regulated partly by TNF signaling. Although TNF-α and EGF alone or in combination with other factors have shown various effects in neuroblastoma, our finding demonstrating that a combination of these factors induced the transdifferentiation of ADRN-type cells to MES-type cells confirms the previously unknown synergistic effects of TNF-α and EGF.

We tested the combination of TNF-α and EGF on other ADRN-type neuroblastoma cell lines (Kelly, NB69, SK-N-BE(2), and SK-N-FI); however, none of them showed obvious changes in the expression of ADRN and MES TF genes and migration abilities, probably due to the context-dependent phenomenon limited to SH-SY5Y cells. Thus, our in vitro findings should be validated in vivo, preferably at the single-cell level in humans.

【Conclusions】

Collectively, we propose a mechanism of neuroblastoma transdifferentiation induced by the combination of TNF-α and EGF, which can be controlled in clinical situations, providing a new therapeutic possibility.