News Release

Title

A mechanism of neuroblastoma tumorigenesis has been revealed

- Polycomb repressive complex 2 was involved and will be a promising therapeutic target -

Key Points

○ Initial events of neuroblastoma tumorigenesis were observed in a mouse model, TH-MYCN mice.

○ Polycomb repressive complex 2 (PRC2) was involved in transcriptomic alterations and essential for the survival of TH-MYCN-derived neuroblastoma cells.

○ Transcriptome of PRC2 target genes were significantly associated with the malignant statuses of human neuroblastomas.

○ Thus, PRC2 will be a promising therapeutic target.

Summary

Postdoctoral researcher Shoma Tsubota and Professor Kenji Kadomatsu (Department of Molecular Biology) at Nagoya University Graduate School of Medicine (Dean: Kenji Kadomatsu, MD, PhD) studied about the tumorigenesis of neuroblastoma, a childhood cancer. The research group revealed the involvement of polycomb repressive complex 2 (PRC2), one of the epigenetic regulators, in the development of neuroblastoma and malignancy in human patients.

In general, gene mutations such as genetic factors, environmental factors (UV or cigarette), and DNA replication errors during cell proliferation of tissue stem cells drive adult tumorigenesis. On the other hand, since neuroblastoma occurs during normal developmental stages, its tumorigenesis is considered to be closely associated with developmental programs. However, gene mutations (such as point mutations) are rare in neuroblastoma and thus the mechanisms of its tumorigenesis are still enigmatic.

The research group utilized TH-MYCN mice, a neuroblastoma mouse model, and established a novel spheroid culture method that can selectively enrich neuroblastoma cells in vitro. Using this method, they successfully captured early events of neuroblastoma tumorigenesis in TH-MYCN mice. They performed global gene expression and epigenetic analysis for the spheres cultured in the established method and revealed the involvement of polycomb repressive complex 2 (PRC2), one of the epigenetic regulators, in the development of neuroblastoma. In addition, they analyzed a publicly available gene expression data set including about 500 neuroblastoma samples. They found that the gene expressions of PRC2 target genes are closely associated with malignant statuses of neuroblastomas (stages and survival). These findings demonstrate the critical role of PRC2 in neuroblastoma tumorigenesis. Therefore, PRC2 would be a potential candidate for the development of novel therapeutic drugs.

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Research Background

Neuroblastoma is a pediatric cancer occurring 2.5-5 cases per 100,000 individuals[1]. More than 90% of patients have an onset of neuroblastoma until 10 years of age. The median age at diagnosis is about 18-months-old; however, patients more than 18-months-old typically show poor prognosis[1]. According to the Japanese investigation, 162 patients (less than 20-year-old) were diagnosed as neuroblastoma in 2014[2]. Survival of neuroblastoma patients with low/intermediate risk is relatively better but those with high risk is less than 50% despite extensive chemotherapy, differentiation therapy, and immune therapy. Therefore, new therapeutic strategies such as molecular targeting drugs are requisite for the improvements of survival ratio and quality of life.

In general, gene mutations such as genetic factors, environmental factors (UV or cigarette), and DNA replication errors during cell proliferation of tissue stem cells drive adult tumorigenesis. On the other hand, since neuroblastoma occurs during normal developmental stages, its tumorigenesis and developmental programs are thought to closely associated. Recent large-scale genome-wide analysis of 240 high-risk neuroblastomas revealed point mutations and small insertions/deletions, and chromosomal alterations including MYCN amplification. However, gene mutations (such as point mutations) are rare in neuroblastoma and thus the mechanisms of its tumorigenesis are still largely unknown. We used TH-MYCN mice, a neuroblastoma model, and investigated the timing and molecular mechanisms of neuroblastoma tumorigenesis.

Research Results

In TH-MYCN mice, an oncogene MYCN is specifically expressed in progenitor cells of sympathetic tissue and drive neuroblastoma tumorigenesis[4]. Although MYCN expression triggered tumorigenesis, its timing and molecular mechanisms were not fully understood. In addition, tumorigenesis starts in a small population; however, it is technically difficult to dissect these cells for further analysis such as global gene expression analysis. To overcome this problem, we established a novel spheroid culture method that can selectively enriches undifferentiated neuroblastoma cells in vitro (Figure 1). Using this method, we addressed the timing and mechanisms of neuroblastoma tumorigenesis.

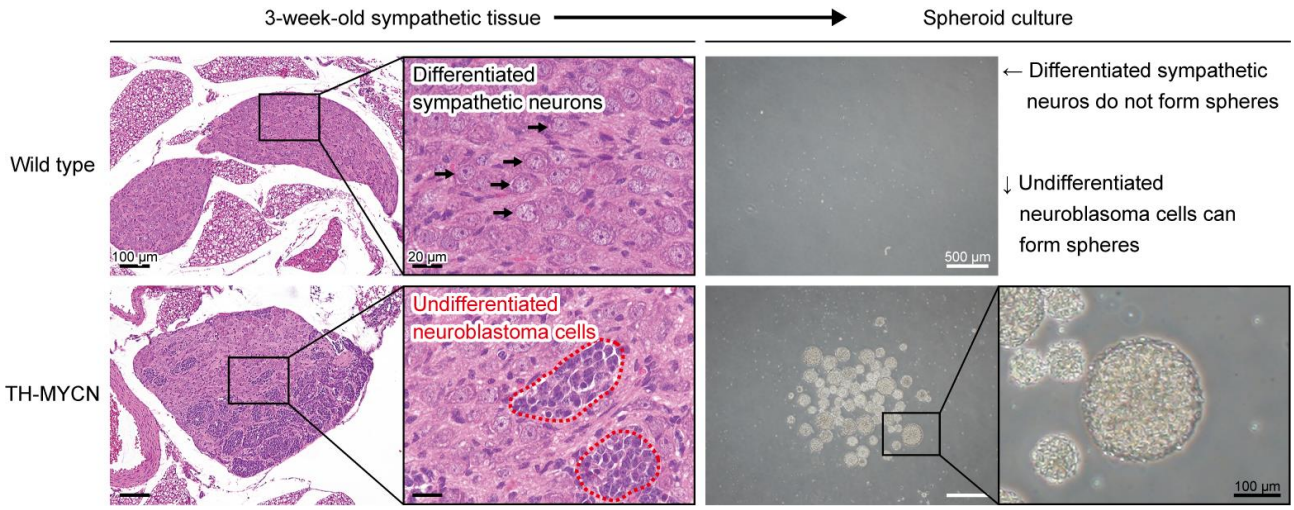

Figure 1. Undifferentiated neuroblastoma cells can form spheres in the novel culture method

To reveal the timing of tumorigenesis, we investigated the expression pattern of MYCN mRNA by in situ hybridization on the tissue sections of embryonic day 13.5 (E13.5), postnatal day 0 and 2-week-old mice. MYCN was expressed in a subset of progenitor cells at E13.5, and the number of MYCN (+) cells increased at postnatal day 0 and 2-wee-old TH-MYCN mice (Figure 2). We dissected tissues from E13.5 and cultured these cells in vitro. Importantly, sphere formation was observed from 50% of E13.5 mice. In addition, these spheres showed tumorigenicity evidenced by subcutaneous transplantation of these cells into wild-type mice. These results indicate that tumorigenesis starts as early as E13.5 in TH-MYCN mice.

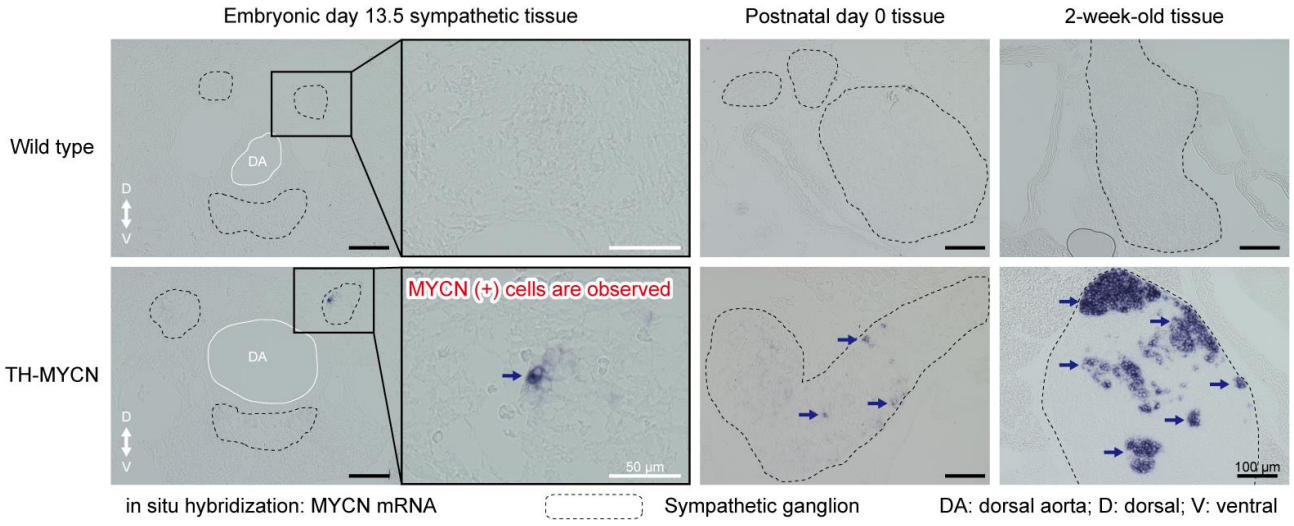

Figure 2. MYCN expression starts as early as E13.5 in TH-MYCN mice

To reveal the molecular mechanisms of tumorigenesis, we performed global gene expression analysis by microarray for the spheres derived from E13.5 wild-type and TH-MYCN mice. Though the analysis, we found that differentially expressed genes included the targets of polycomb repressive complex 2 (PRC2), an epigenetic regulator. PRC2 comprises Ezh2, Eed, Suz12 and catalyzes tri-methylation of histone H3 at lysine 27 (H3K27me3) to suppress the expressions of its target genes. Interestingly, the expressions of PRC2 components, Ezh2, Eed,

and Suz12 were not changed but its target genes were significantly downregulated (left panel in Figure 3). In addition, we observed an increase in H3K27me3 modifications at the genomic regions of PRC2 target genes (right panel in Figure 3). These results indicate that PRC2 alters epigenetic status of its target genes and suppress their expressions in TH-MYCN mice.

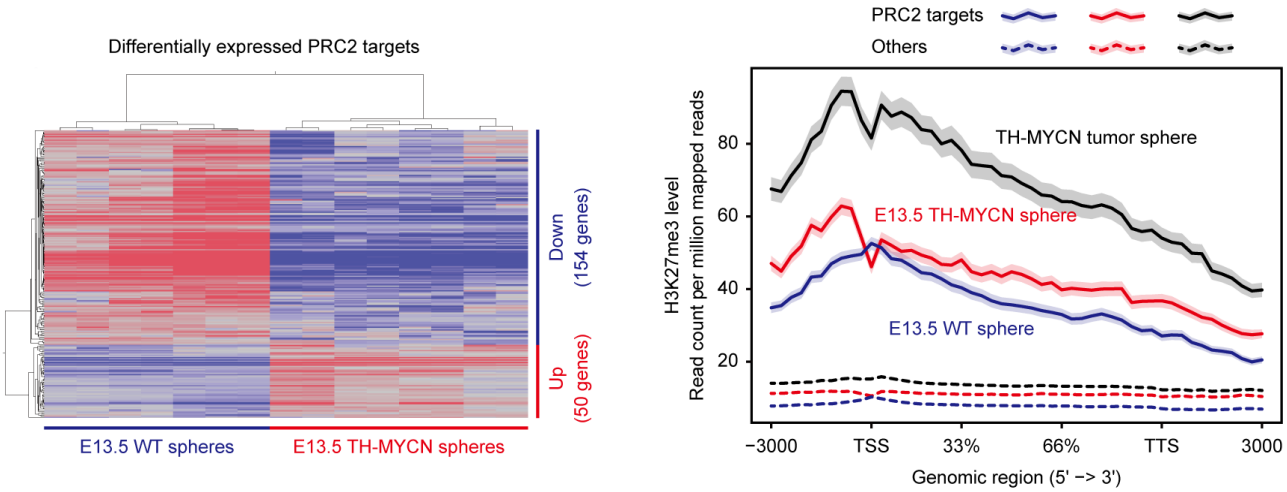

Figure 3. PRC2 target genes were downregulated (left panel) and H3K27me3 levels were increased at the genomic region of PRC2 targets in TH-MYCN spheres (right panel).

Ezh2 is a histone methyltransferase and responsible for the function of PRC2. Next, we examined whether Ezh2 (including PRC2) is essential for survival of neuroblastoma cells. Short-hairpin RNAs targeting Ezh2 mRNA and Ezh2 inhibitor EPZ-6438 were used to inhibit the function of Ezh2. Importantly, both significantly suppressed TH-MYCN sphere formation in vitro (for EPZ-6438, Figure 4). We also confirmed that PRC2 target genes were de-repressed by the treatment of spheres with Ezh2 inhibitor. Moreover, administration of EPZ-6438 significantly suppressed in situ tumor growth in TH-MYCN mice suggesting that Ezh2 supports the survival of neuroblastoma cells.

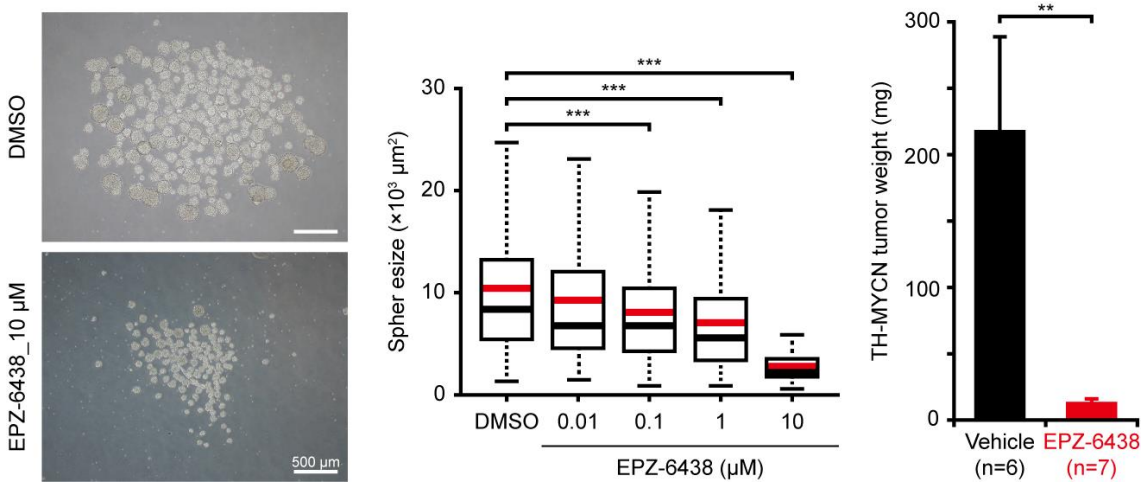

Figure 4. Ezh2 inhibitor EPZ-6438 suppressed sphere formation in a dose-dependent manner and suppressed in situ tumor growth in TH-MYCN mice

Finally, we used publicly available gene expression data including about 500 neuroblastoma samples to investigate the association between the expression of PRC2 targets and clinical status of human neuroblastoma. Importantly, the expression of PRC2 target genes were strongly and negatively associated with MYCN expression, which is consistent with the result we observed in TH-MYCN mice. Moreover, the expression of PRC2 target genes were significantly lower in malignant neuroblastomas (stage 4 and MYCN-amplified) and are associated with poor prognosis (Figure 5). These results indicate that the expression pattern of PRC2 target genes, in other words PRC2-mediated transcriptomic regulation, is strongly associated with malignancies of human neuroblastoma.

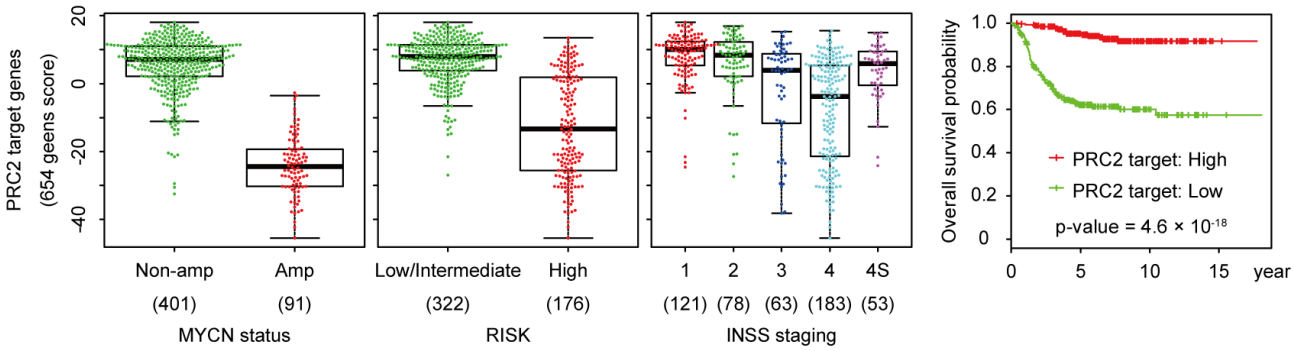

Figure 5. Expression score of PRC2 target genes are negatively associated with malignant statuses (MYCN-amp, High-risk, and Stage 4) and predicted patient prognosis

Research Summary and Future Perspective

Our study revealed the involvement of PRC2 in tumorigenesis of MYCN-driven neuroblastoma and clinical outcome of human patients. However, it is still unknown how PRC2-mediated transcriptome is regulated and which molecules regulates this process. This study mainly focused on MYCN-driven neuroblastomas and thus neuroblastoma driven by other factors will be investigated in future. However, this study opens new therapeutic avenue targeting PRC2-mediated transcriptomic alterations.

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Publication

Shoma Tsubota, Satoshi Kishida, Teppei Shimamura, Miki Ohira, Satoshi Yamashita, Dongliang Cao, Shinichi Kiyonari, Toshikazu Ushijima, Kenji Kadomatsu. PRC2-mediated transcriptomic alterations at the embryonic stage govern tumorigenesis and clinical outcome in MYCN-driven neuroblastoma. *Cancer Research*, published online on August 14, 2017.
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Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf//Cancer_R_20170907.pdf