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Review

Analysis of Asymmetric Cell Division Using Human Neuroblastoma Cell Lines as a Model System

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Abstract: Neuroblastoma is one of the most common childhood solid tumors and develops from neural stem cells that normally comprise the embryonic structure termed the neural crest. Human neuroblastoma cell lines have special properties as they exhibit cell growth and are induced to become mature neurons by drugs such as retinoid. Therefore, we examined asymmetric cell division (ACD) using human neuroblastoma cells as an ACD model, and confirmed that ACD in human cancer cells is evolutionally conserved. Furthermore, we demonstrated that MYCN is involved in cell division fate. We introduce the brief history of ACD study using neuroblastoma cell lines and discuss why human neuroblastoma cells are an ideal model system for clarifying the mechanism of ACD.

Keywords: neuroblastoma; asymmetric cell division; centrosome; MYCN; TRIM32

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1. Introduction

Neuroblastoma is one of the most typical solid tumors among childhood cancers and exhibits a wide clinical range [1–3]. Patients can be broadly divided into three patterns (low-risk, medium-risk, and high-risk groups) from a biological and clinical point of view [1–3]. Although minimum treatment may be sufficient for low-risk patients, high risk patents have poor results in spite of aggressive treatment. Among several biological features, amplification of the *MYCN* gene is known to correlate with its prognosis and malignancy. In fact, approximately 20% of neuroblastomas have *MYCN* gene amplification. Recent studies showed that *MYCN* not only exhibits oncogenic activity, but also plays a role in the self-renewal of normal neural stem cells and progenitor cells [4–7].

Neuroblastoma is believed to be derived from the multipotent neural crest cells that make up the structure of the embryo [8]. The neural crest is composed of a cell population that produce cell lineage such as Schwann cell, melanocyte, smooth muscle, peripheral neurons, and glia [8]. Therefore, neuroblastoma cells are suspected to be similar in nature to cancer stem cells due to their pluripotent function.

Cancer stem cells are thought to exhibit asymmetric cell division (ACD), which results in tumor cell heterogeneity [9,10]. ACD is a originate physiological process to maintain the stem cell pool and differentiated cell pool through a single cell division. Recent studies showed that the imbalance between self-renewal and differentiation causes the appearance of abnormal stem cells, leading to tumorigenesis in the *Drosophila* neuroblast population [11–13]. Thus, ACD is a strategy for producing many cancer stem cells and differentiated cancer cells. We used a series of cultured human neuroblastoma cells as a model system to investigate the underlying mechanism of ACD [14–17].

2. Discovery of ACD in Human Neuroblastoma Cells

ACD studies were originally demonstrated using model organisms, such as nematode embryos [18,19], *Drosophila* neuroblasts [20], and *Drosophila* male germ stem cells [21]. These genetic studies showed that the mechanism of ACD is highly conserved. Thereafter, evidence supporting asymmetric cell division in mammalian stem cells, such as muscle [22], skin [23], gut [24], mammary glands [25], the hematopoietic system [26], and developing mouse brain [27,28], was reported.

As mentioned above, human neuroblastoma cell lines have special properties as they exhibit cell growth and are induced to become mature neurons by drugs such as retinoid [29]. In addition, neuroblastoma is a common childhood cancer that may develop at the fetal development stage when a large number of stem cells display ACD [17]. Therefore, we investigate ACD using human neuroblastoma cells as a model [14]. As a result, although the cell lines with *MYCN* amplification exhibited symmetric cell division (self-renewal division), the cell lines without *MYCN* amplification exhibited ACD (Figure 1). In addition, comparison of ACD studies using these organisms and model systems demonstrated that ACD in neuroblastoma cells is evolutionarily conserved [14].

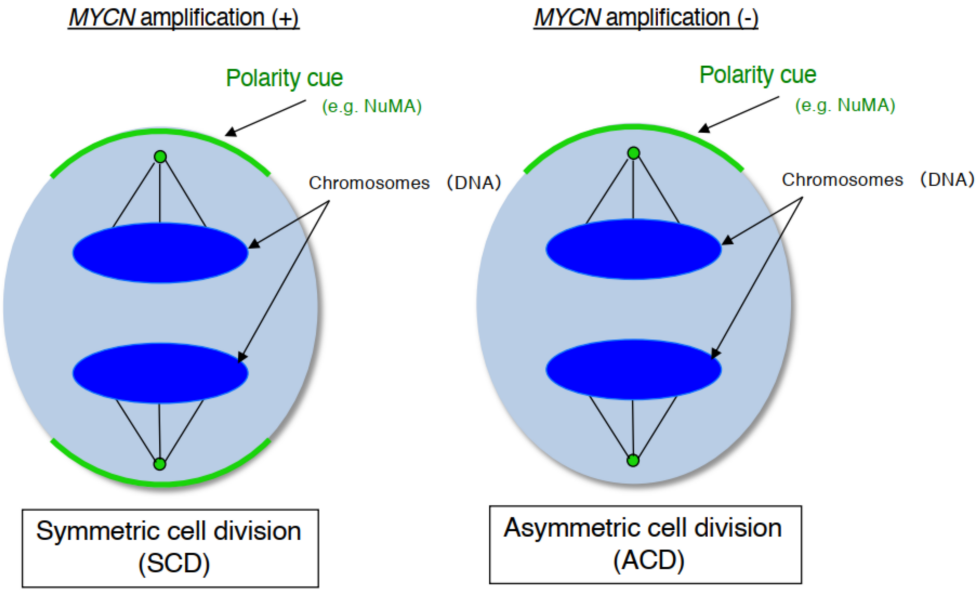

Figure 1. Detection of NuMA-based asymmetric cell division in human neuroblastoma cells.

During late mitosis (anaphase), spindle pole-localized NuMA also localizes to the cell cortex as a polarity cue, and the cell cortex localized-NuMA ensures proper polarity division. Neuroblastoma cells with *MYCN* amplification exhibit symmetric NuMA cortex-based cell division, whereas those without *MYCN* amplification exhibit asymmetric NuMA cortex-based cell division (the ACD ratio is approximately 20%).

3. MYCN Regulates Cell Division Fate

MYCN is a typical transcriptional factor that belongs to the MYC family (c-MYC, L-MYC) [30]. As mentioned above, recent studies have reported that MYCN not only exhibits oncogenic activity, but also plays a central role in the self-renewal of normal neural stem and progenitor cells [31].

In our experiments, the gene expression level of *MYCN* influenced the control of cell division fate. The overexpression of *MYCN* induced symmetric cell division (SCD) (self-renewal division), whereas decreased expression of *MYCN* caused ACD [14]. Moreover, when its leucine zipper domain was deleted, the MYCN mutant no longer induced self-renewal division. This suggested that the transcriptional activity of MYCN is necessary for inducing SCD in human neuroblastoma cells [14]. Although the specific transcriptional tar-

get(s) of MYCN currently remain unclear except for the high mobility group A1 (HMGA1) oncogene [32], several molecular pathways have been identified in MYCN-mediated cell division fate (Figure 2) [17].

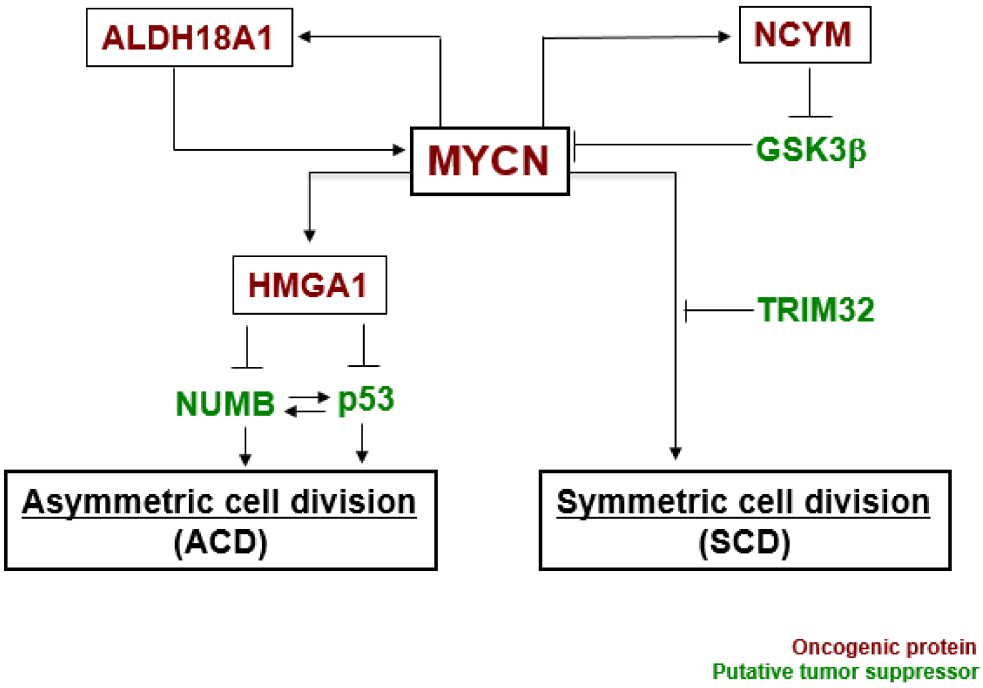

Figure 2. The pathways of MYCN-mediated cell division fate.

MYCN and ALDH18A1 form a positive feedback loop for their transcriptional expression [33]. This positive feedback loop effectively induces symmetric cell division (SCD). NCYM inhibits GSK3β–mediated phosphorylation of MYCN, resulting in induction of SCD [34,35]. HMGA1 is a transcriptional target of MYCN [32]. HMGA1 inhibits the expression of NUMB [36] and p53 [37]. NUMB and p53 induce asymmetric cell division (ACD) [25,38]. Lastly, TRIM32 facilitates proteasomal degradation of MYCN to induce asymmetric cell division (ACD) [15]. Thus, MYCN-dependent tumor cells exhibit symmetric cell division (SCD) and the degradation of MYCN causes asymmetric cell division (ACD).

4. Induction of ACD in Human Neuroblastoma Cells

Based on the results of above previous studies, we searched for a molecule that degrades the MYCN protein and induces asymmetric division, focusing on TRIM32 (Tripartite motif-containing 32) by analogy. Previous studies reported that *TRIM32*, an ortholog of *Drosophila melanogaster*, *Brat*, which controls ACD as a determinant of neuron, suppresses *Drosophila* MYC (dMYC) function in the neuroblasts of flies [39]. Moreover, mouse TRIM32 was reported to have ubiquitin ligase activity, and facilitated the degradation of the c-MYC oncoprotein during neurogenesis [40]. In neuroblastoma cells, TRIM32 associated with MYCN at the spindle pole during mitosis and facilitated the degradation of MYCN protein as an ubiquitin ligase [15]. In addition, when *TRIM32* was overexpressed, ACD was detected in human neuroblastoma cells, as expected (Figure 3) [15].

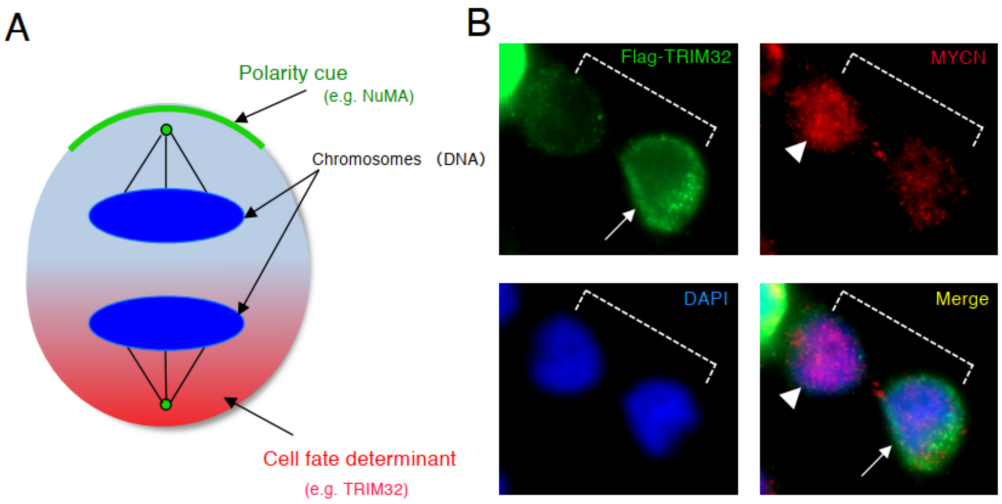

Figure 3. Detection of NuMA/TRIM32-based asymmetric cell division in human neuroblastoma cells (**A**) Overexpression of TRIM32 can induce asymmetric cell division. During late mitosis (anaphase), NuMA leads to complete polarity cell division as a cell polarity cue, whereas TRIM32 functions in asymmetric cell division as a cell fate determinant. (**B**) Representative image of asymmetric cell division in SK-N-DZ neuroblastoma cells with *MYCN* amplification transfected with the Flag-*TRIM32* expression vector. *TRIM32* encodes a ubiquitin ligase for MYCN degradation.

5. Centrosome Inheritance in ACD

During the process of ACD, there is some controversy regarding the patterns of centrosome inheritance, as different results have been reported among studies using different model systems [41]. Recent results are summarized in Table 1. In the G1 phase of the cell cycle, the centrosome consists of one mother centriole and one daughter centriole. During the replication of the centriole, there are three generations of centrioles: an older mother, a younger mother, and two new daughter centrioles. The centrosome with the old mother's centriole is termed the mother centrosome. In the case of male germline stem cells of *Drosophila melanogaster,* [21], mouse neural progenitor cells [28], and mouse ES cells [42], the mother centrosome remains in the stem cell and the daughter centrosome is inherited by the daughter cell that has differentiation potential. On the other hand, in the case of *Drosophila melanogaster* neuroblasts [43], female germline stem cells [44] and human neuroblastoma cells [14], the mother centrosome does not stay in the daughter cells with self-renewal ability, but is inherited by those with differentiation potential.

Table 1. A list of asymmetric centrosome segregation in asymmetric cell division.

Model	Pattern of Centrosome Inheritance	Reference
Drosophila male germ stem cells	Stem cells inherit the mother centrosome	[21]
Drosophila female germ stem cells	Stem cells inherit the daughter centrosome	[44]
Drosophila neuroblasts	Stem cells inherit the daughter centrosome	[43]
Mouse neural progenitors	Stem cells inherit the mother centrosome	[28]
Mouse ES cells	Stem cells inherit the mother centrosome	[42]
Human neuroblastoma cells	NuMA+ cells inherit the daughter centrosome	[14]

Of note, in human glioblastoma cells, the result was opposite when compared with that from neuroblastoma cells (Koguchi and Izumi, unpublished work). Glioblastoma originates from the central nervous system, whereas neuroblastoma is from the peripheral

nervous system. Therefore, the mechanism of centrosome inheritance in stem cells may differ between the central and peripheral nervous systems. Alternatively, "centrosome inheritance instability" may be characteristic of cancer (stem) cells.

6. Conclusions

Why do cultured neuroblastoma cells display ACD? As mentioned above, many cultured neuroblastoma cells exhibit a special ability to proliferate and differentiate. Previous studies have shown that human neuroblastoma cell lines are classified into three different cell phenotypes with different potencies: neuroblastoma type (N-type), substrate adherent type (S-type), and intermediate type (I-type) [8]. Based on its morphological, biochemical, and growth characteristics, I-type was approved as a neuroblastoma stem cell due to its differentiation and malignant potential [8]. Furthermore, as described above, neuroblastoma is typical childhood cancer that may have many stem cells exhibiting ACD that were generated in the developmental stage [17].

Although this review presents the intrinsic factors of ACD, further studies are necessary for clarify the extrinsic factors of ACD such as the tumor microenvironment. For this purpose, the neuroblastoma culture system is advantageous in that it is possible to build a variety of extracellular environments in vitro.

Human neuroblastoma cultured cells are suitable model system for providing insight into the mechanisms underlying ACD. We hope that many ACD studies will employ cultured neuroblastoma cells.

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