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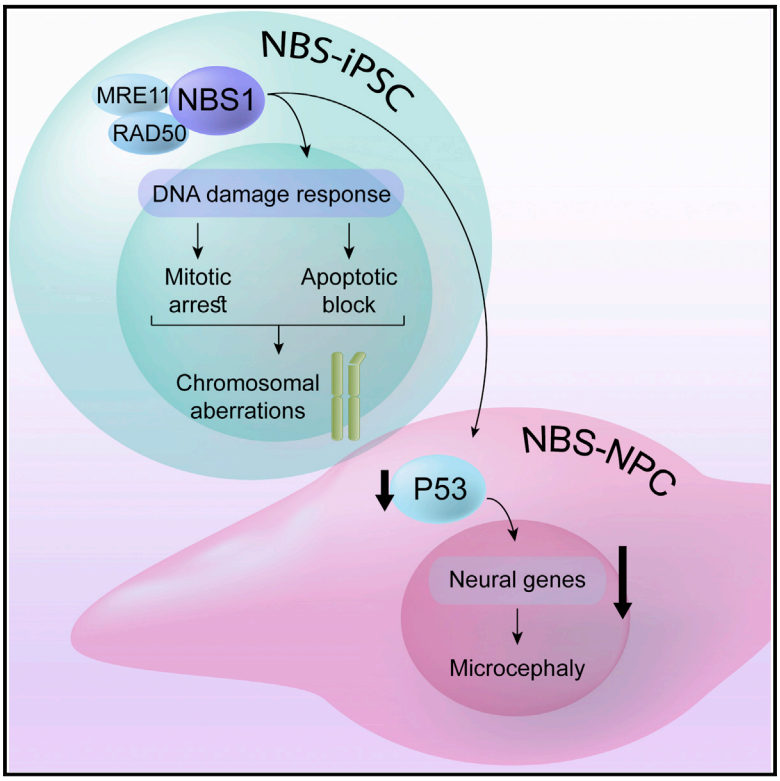
Chromosomal Instability and Molecular Defects in Induced Pluripotent Stem Cells from Nijmegen Breakage Syndrome Patients

Graphical Abstract



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In Brief

Halevy et al. describe molecular defects in induced pluripotent stem cells derived from Nijmegen breakage syndrome (NBS) patients and find mitotic and apoptotic blocks leading to chromosomal instability. NBS-neural progenitor cells reveal downregulation of neural genes correlated with low P53 levels, which may underlie severe microcephaly observed in patients.

Highlights

- NBS-iPSC reprogramming shows selection for karyotypically normal NBS fibroblasts
- NBS-iPSCs display delayed response to DSBs and acquire chromosomal aberrations
- NBS-iPSCs exhibit mitotic arrest and apoptotic block
- NBS-NPCs display downregulation of neural genes correlated with low levels of P53

Accession Numbers

GSE83686

Halevy et al., 2016, Cell Reports *16*, 2499–2511
August 30, 2016
http://dx.doi.org/10.1016/j.celrep.2016.07.071

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Chromosomal Instability and Molecular Defects in Induced Pluripotent Stem Cells from Nijmegen Breakage Syndrome Patients

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SUMMARY

Nijmegen breakage syndrome (NBS) results from the absence of the NBS1 protein, responsible for detection of DNA double-strand breaks (DSBs). NBS is characterized by microcephaly, growth retardation, immunodeficiency, and cancer predisposition. Here, we show successful reprogramming of NBS fibroblasts into induced pluripotent stem cells (NBS-iPSCs). Our data suggest a strong selection for karyotypically normal fibroblasts to go through the reprogramming process. NBS-iPSCs then acquire numerous chromosomal aberrations and show a delayed response to DSB induction. Furthermore, NBS-iPSCs display slower growth, mitotic inhibition, a reduced apoptotic response to stress, and abnormal cell-cycle-related gene expression. Importantly, NBS neural progenitor cells (NBS-NPCs) show downregulation of neural developmental genes, which seems to be mediated by P53. Our results demonstrate the importance of NBS1 in early human development, shed light on the molecular mechanisms underlying this severe syndrome, and further expand our knowledge of the genomic stress cells experience during the reprogramming process.

INTRODUCTION

Nijmegen breakage syndrome (NBS) is a rare autosomal recessive disease that results from a null mutation in the *NBS1* gene (Carney et al., 1998; Varon et al., 1998). NBS1 together with MRE11 and RAD50, form the MRN complex that senses DNA double-strand breaks (DSBs) and starts the DNA repair cascade (Carney et al., 1998). Due to the critical role of NBS1 in the cellular DNA damage response (DDR), affected children with the disease have an exceptionally high risk of developing cancer at a young age (van der Burgt et al., 1996). This is accompanied with other cardinal phenotypes of the syndrome such as progressive microcephaly with distinct facial features, growth delay, immunodeficiency, and premature ovarian failure in girls. Neurocognitive abilities vary among patients but mental retardation usually progresses during childhood (Chrzanowska et al., 2012; van der Burgt et al., 1996).

Previous work on cells derived from NBS patients suggested impairments in cell cycle and regulation of apoptosis (Buscemi et al., 2001; Hou et al., 2012; Jongmans et al., 1997; Porcedda et al., 2006; Rogoff et al., 2004). Moreover, NBS1 was shown to modulate cellular P53 levels through kinase activation (Jongmans et al., 1997; Lee and Paull, 2005). In the past, murine models were created to study NBS; however, null mutations of *NBS1* in mice resulted in early embryonic lethality (Zhu et al., 2001). A mouse model with a truncated NBS1 protein was also created; this model also failed to recapitulate the phenotypes seen in humans, as it showed no immunodeficiency and no increased in cancer development, and the animals were fertile (Williams et al., 2002). In order to study the importance of NBS1 in early development and the features of the disease that lead to the severe phenotypes seen in affected individuals, a different model system must be used. Human embryonic stem cells (ESCs) serve as a great tool in modeling developmental diseases. However, due to the rarity of the syndrome, we do not know of any ESCs with NBS available for research. To overcome this obstacle, we utilized primary NBS fibroblasts that can be reprogrammed into induced pluripotent stem cells (iPSCs) and thus be used to model NBS or other syndromes caused by aberrant DDR. Reprogramming of somatic cells into pluripotent cells has become a routine procedure in many labs; however, some studies have suggested that lack of different DDR proteins renders this process inefficient (Nayler et al., 2012; Raya et al., 2009; Tilgner et al., 2013; Yung et al., 2013). Moreover, the reprogramming process coupled with a DDR mutation and prolonged culturing induces stress to the genomic integrity of these cells and so must be closely monitored.