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Novel 1p tumour suppressor Dnmt1-associated protein 1 regulates MYCN/ataxia telangiectasia mutated/p53 pathway

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Abstract Neuroblastoma (NB) is a paediatric solid tumour which originates from sympathetic nervous tissues. Deletions in chromosome 1p are frequently found in unfavourable NBs and are correlated with v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (*MYCN*) amplification; however, it remains to be elucidated how the 1p loss contributes to MYCN-related oncogenic processes in NB. In this study, we identified the role of Dnmt1-associated protein 1 (DMAP1), coded on chromosome 1p34, in the processes.
We studied the expression and function of DMAP1 in NB and found that low-level expression of DMAP1 related to poor prognosis, unfavourable histology and 1p Loss of heterozygosity (LOH) of primary NB samples. Intriguingly, DMAP1 induced ataxia telangiectasia mutated (ATM) phosphorylation and focus formation in the presence of a DNA damage reagent, doxorubicin. By DMAP1 expression in NB and fibroblasts, p53 was activated in an ATM-dependent manner and p53-downstream pro-apoptotic Bcl-2 family molecules were induced at the mRNA level, resulting in p53-induced apoptotic death. *BAX* and $p21^{Cip1/Waf1}$ promoter activity dependent on p53 was clearly up-regulated by DMAP1. Further, MYCN transduction in *MYCN* single-copy NB cells accelerated doxorubicin (Doxo)-induced apoptotic cell death; MYCN is implicated in DMAP1 protein stabilisation and ATM phosphorylation in these situations. DMAP1 knockdown attenuated MYCN-dependent ATM phosphorylation and NB cell apoptosis. Together, DMAP1

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appears to be a new candidate for a 1p tumour suppressor and its reduction contributes to NB tumourigenesis via inhibition of MYCN-related ATM/p53 pathway activation.

1. Introduction

Genetic and molecular analyses have indicated various types of deletions of the short arm of chromosome 1 (1p) in a broad range of human malignant tumours, including neuroblastoma (NB) and others [1–5]. It has been suggested that this genomic region harbours several tumour suppressor genes and that additive effects of loss of those tumour suppressors on tumourigenesis exist in several '1p loss malignant tumours'.

NB is the second most common paediatric solid malignant tumour derived from sympathetic nervous tissues. Extensive cytogenetic and molecular genetic studies have identified that genetic abnormalities, such as loss of the short arm of 1p, 11q and 14q; amplification of *MYCN*; and allelic gain of 11p and 17q, are frequently observed [1]. Deletion of the 1p region is highly correlated with both *MYCN* amplification and an adverse patient outcome, indicating the presence of several tumour suppressor genes (TSGs) within this region [6]. NB tumours with *MYCN* in a single copy had preferentially lost the lp36 allele and these tumours also had a very distal commonly deleted region; in contrast, all *MYCN*-amplified NBs had larger 1p deletions, extending from the telomere to lp31 [7]. The extent of deletion or LOH was identified in 184 primary NBs; in 80%, the 1p deletion extended from the telomere to lp31 [8]. Given the tendency of large, hemizygous 1p deletions in *MYCN*-amplified NBs, alternative hypotheses for tumour suppression are: (1) an additional, *MYCN*-associated TSG in the 1p region; (2) suppression of TSG expression from a hemizygous allele due to epigenetic modifications except for imprinting, e.g. miRNAs and non-coding RNAs; (3) haplo-insufficiency-based suppression accounting for the rarity of 1p homozygous deletions [9].

Dnmt1-associated protein 1 (DMAP1) was originally identified as a molecule interacting with DNMT1 and was demonstrated to co-localise with PCNA and DNMT1 at DNA replication foci during the S phase [10]. Previously, we reported that Dmap1 participates in DNA repair and transformation of mouse embryonic fibroblasts (MEFs). Dmap1 was recruited to the damaged sites, formed complexes with γ-H2AX and directly interacted with Proliferating Cell Nuclear Antigen (Pcna); inhibition of this binding impaired the accumulation of the Pcna-Caf-1 complex at damaged sites and resulted in DNA breaks [11]. In addition, Penicud and Behrens reported that DMAP1 promotes ataxia telangiectasia mutated (ATM) recruitment and focus formation at damaged sites. These results suggest that DMAP1 is involved in the DNA damage response (DDR) [12]. Interestingly, DMAP1 gene is coded in 1p34 and the region that is frequently deleted in NB tumours with 1p LOH [8,9]. These results prompted us to study the expression level of DMAP1 in neuroblastoma samples and its functional role in tumourigenesis.

In the present report, for the first time, we found that DMAP1 is a novel 1p tumour suppressor and DMAP1 has an indispensable role in MYCN-related ATM/p53 pathway activation. Downregulation of DMAP1 seems to be a result of MYCN-induced stress and an important mechanism for NB tumourigenesis.

2. Materials and methods

2.1. Cell culture

Human NB cell lines were obtained from official cell banks (RIKEN Bioresource Cell Bank, Tohoku University Cell Resource Center, and the American Type Culture Collection) and were cultured in RPMI1640 or Dulbecco's modified Eagle's medium (Wako, Osaka, Japan) supplemented with 10% heat-inactivated foetal bovine serum (Invitrogen, Carlsbad, CA, United States of America (USA)) and 50 μg/ml penicillin/streptomycin (Sigma–Aldrich, St. Louis, MO, USA) in an incubator with humidified air at 37 °C with 5% CO_2. ATM kinase inhibitor, KU-55933 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was dissolved in DMSO to make stock solutions of 20 mM.

2.2. Lentiviral production and infection for over-expression and knockdown of genes

For the over-expression of mouse Dmap1 and human DMAP1, cDNAs were subcloned into lentiviral vector pHR-SIN-CSGW [13]. For shRNA-based knockdown experiments, pLKO.1 puromycin-based lentiviral vectors containing five sequence-verified shRNAs targeting human DMAP1 (RefSeq NM_019100.4, NM_001034024.1, NM_001034023.1) were obtained from the MISSION TRC-Hs 1.0 Human, shRNA library (Sigma–Aldrich). We checked DMAP1 knockdown by five lentivirus-produced shRNAs (clones: TRCN0000021744-21748) and used at least two shRNAs for experiments. Lentiviral production, infection and confirmation of infection efficiency were performed as described previously [13].

2.3. Antibodies

Antibodies against p53 (DO-1) and MYCN (rabbit polyclonal, C-19) were purchased from Santa Cruz Biotechnology. Antibodies against p53Ser15-P (rabbit