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The fusion partner specifies the oncogenic potential of *NUP98* fusion proteins

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ABSTRACT

NUP98 is among the most promiscuously translocated genes in hematological diseases. Among the 28 known fusion partners, there are two categories: homeobox genes and non-homeobox genes. The homeobox fusion partners are well-studied in animal models, resulting in HoxA cluster overexpression and hematological disease. The non-homeobox fusion partners are less well studied. We created transgenic animal models for three *NUP98* fusion genes (one homeobox, two non-homeobox), and show that in this system, the *NUP98*-homeobox fusion promotes self-renewal and aberrant gene expression to a significantly greater extent. We conclude that homeobox partners create more potent *NUP98* fusion oncogenes than do non-homeobox partners.

1. Introduction

Chromosomal translocations in human leukemia are a source of novel oncogenes termed fusion genes, which encode fusion proteins combining the properties of two distinct proteins. Some genes are involved in multiple oncogenic fusion proteins with distinct partner genes, indicating that the properties of that gene are generally oncogenic if deregulated, or that there are multiple mechanisms by which oncogenic fusion proteins involving the gene can act. The *Nucleoporin 98* (*NUP98*) gene encodes a protein which is normally involved in nuclear transport, but is also recurrent in many different chromosomal translocations in myelodysplastic syndrome (MDS) and acute leukemia [1]. It is among the most promiscuous known fusion gene partners, having been identified in fusion genes with 28 distinct partner genes. The estimated frequency of *NUP98* translocations in human leukemia has been recently revised upward, with modern molecular techniques leading to superior detection of these translocations [2,3]. Further, the NUP98-JARID1A fusion represents a functionally distinct subclass of AML, suggesting that other NUP98 fusions may do the same [2]. Understanding the leukemic activity of the various *NUP98* fusions is therefore of increasing importance.

NUP98 fusion partner genes can be broadly broken into two groups: homeobox genes and non-homeobox genes. Homeobox genes are transcription factors defined by the conserved "homeodomain" DNA-binding domain, and this DNA-binding domain is conserved in the fusion proteins with NUP98, of which there are at least ten [1]. This retention of the DNA-binding domain suggests a transcriptional regulatory mechanism for the NUP98-homeobox fusion proteins. Indeed in the case of the NUP98-HOXA9 (NHA9) fusion, this has been directly shown [4]. For the non-homeobox fusion proteins, of which there are at least eighteen [1], an overarching mechanism has been slower to emerge. Recent work has suggested that regulation of gene expression by chromatin reading and/or writing via indirect DNA binding is the mechanism for at least some non-homeobox fusion partners [1,5,6], although not all eighteen have the requisite domains for this activity.

We have previously reported the generation of the *NUP98-HOXD13* (*NHD13*) transgenic mouse [7]. Created using the cloned fusion gene from a human MDS under a transgenic vav promoter [8], the *NHD13* mouse develops an MDS by the age of five months (100% penetrance) and an acute leukemia from the age of six months (60% penetrance). The only other reported example of a

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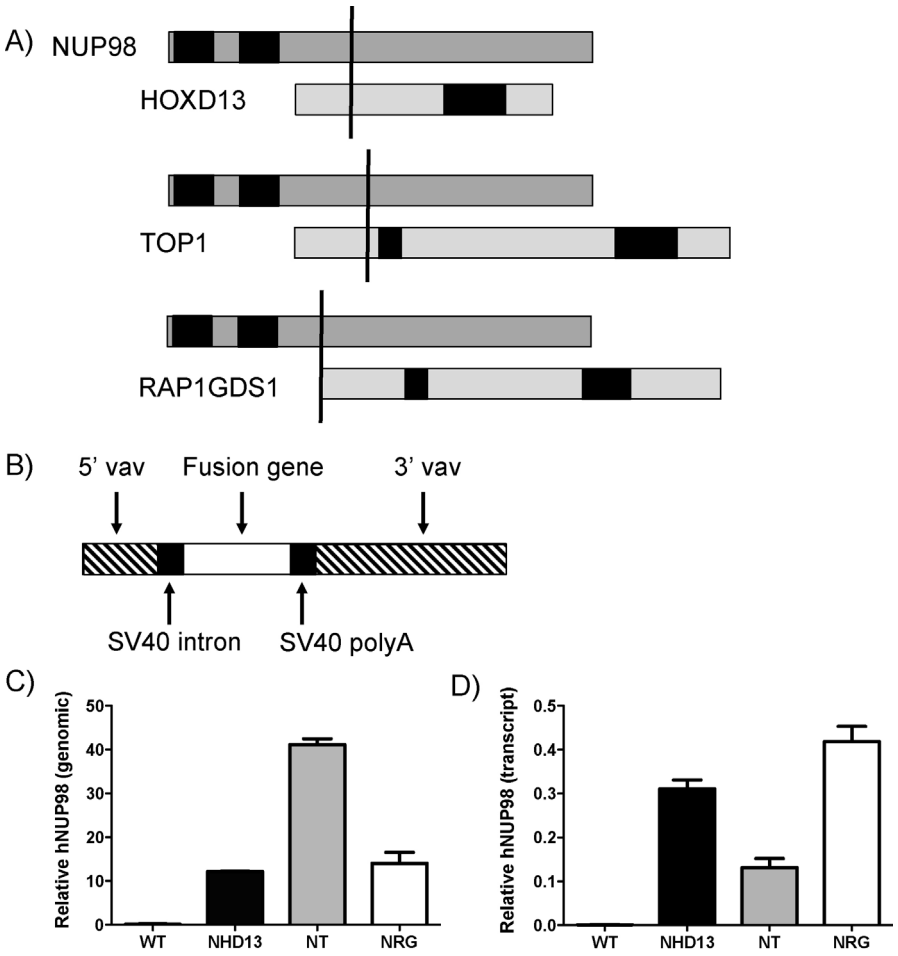

Fig. 1. Creation of NT and NRG transgenic mice. (A) Schematic showing the retained domains of each fusion partner gene. In each case the upper gene is NUP98, with the GLFG repeat domains shown in black, and the lower gene is the fusion partner. In all fusions, the 5′ portion of NUP98 is fused to the 3′ port ion of the partner. For HoxD13 the black box represents the homeodomain, for Top1 the boxes represent (left to right) the core, linker and catalytic domains, and for Rap1GDS1 the black boxes represent the armadillo domains. The vertical black line represents the fusion point. (B) Structure of the vav vector used to generate the transgenic mice. (C) Real-time PCR data indicating the relative signal obtained for a transgene-specific NUP98 amplicon from bone marrow obtained from each of the three mouse lines and wild type mice. (D) Real-time PCR data indicating the signal (relative to HPRT signal) obtained for a transgene-specific NUP98 amplicon from LK cells obtained from each of the three mouse lines and wild type mice.

transgenic *NUP98* fusion is also a *NUP98*-homeobox fusion; the *NHA9* transgenic mouse is also reported to develop acute myeloid leukemia (AML), albeit at a lower penetrance and later age (22% by 15 months) [9]. No transgenic models of *NUP98*-non-homeobox fusion genes have been reported, but there are numerous reports of retrovirally-transduced bone marrow models of these genes, which generally result in myeloproliferative neoplasms or AML [5,6,10].

We sought to investigate the relative oncogenic activity of homeobox and non-homeobox *NUP98* fusion genes using similar techniques. To this end, we selected three NUP98 fusion genes to study: *NUP98-HOXD13* (*NHD13*) [11], *NUP98-TOP1* (*NT*) [12] and *NUP98-RAP1GDS1* (*NRG*) [13]. All of these fusion genes retain the 5′ portion of *NUP98*, including the FG repeats responsible for karyopherin docking during nuclear transport [14] (Fig. 1A). The 3′ portion of *HOXD13* that is retained includes the homeodomain, a DNA binding domain. The 3′ portion of *TOP1* (*Topoisomerase 1*) that is retained includes the core, linker and catalytic domains used in the protein's normal function of unwinding DNA superstructures [15]. The 3′ portion of the *NRG* fusion retains the entirety (bar the first methionine) of *RAP1GDS1* (*RAP1 GTP-GDP dissociation stimulator 1*), including the armadillo domain. *RAP1GDS1* encodes a protein known as smgGDS, which is involved in guanine nucleotide exchange activity [16]. We created transgenic mouse models expressing *NT* and *NRG* from the same vav promoter used in the creation of the *NHD13* mouse. We compare phenotype, aberrant gene expression profiles and abnormal self-renewal activity in each of these models to determine the relative oncogenic potency of each of these genes.

2. Materials and methods

2.1. Generation of transgenic mice

The *NUP98-HOXD13* mice have been described previously (Lin et al., 2005). We used the pZVNHD13 vector used in the generation of these mice as the basis for generating the *NUP98-TopoisomeraseI* (*NT*) and *NUP98-RAP1GDS1* (*NRG*) mice. Full-length *NT* and *NRG* cDNAs were PCR amplified and cloned into the pZVNHD13 vector, replacing the *NHD13* sequence in the vector. These resultant vectors (pZVNT and pZVNRG) were sequenced to verify the constructs. The pZVNT and pZVNRG plasmids were digested with PmeI, and the insert containing 5′ and 3′ vav regulatory elements and the respective fusion gene cDNA were purified by agarose gel electrophoresis and Qiagen gel purification, using the manufacturer's recommendations. The construct was microinjected into zygotes obtained from C57Bl6 mice. Founders were identified using a human *NUP98* probe, and offspring were genotyped by PCR amplification of the respective transgene from tail biopsy DNA. Lines were maintained by mating with wild-type C57bl6 mice. All animal experiments were approved by the Animal Care and Use Committee at NCI or the Animal Ethics Committee at Monash University.