The cell cycle protein Nop52 is a candidate target autoantigen

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Background: Indirect immunofluorescence (IIF) on HEp-2 cells has permitted the detection of autoantibodies (aabs) targeting autoantigens that are transiently expressed in different phases of the cell cycle such as proliferating cell nuclear antigen (PCNA), centromere protein F (CENP-F) and the mitotic spindle apparatus. Nop52 was originally identified and cloned with the aid of human aabs from a male bone marrow transplant patient. Nop52 belongs to a class of nucleolar proteins, the nucleolar organizer region (NOR) proteins, along with Nucleolin and B23 (numatrin; a known human autoantigen) and few others that are involved in processing of the 27S pre-ribosomal RNA macromolecule to the 25S and 5.8S rRNA. Transfection experiments confirmed that the cDNA encodes a nucleolar protein that accumulates in the granular domain of nucleoli and at the periphery of the chromosomes. In nucleoli, it mainly co-localizes with B23 involved in the late pre-rRNA processing. The objective was to identify the autoantigen of patient's sera that presented a cell-cycle dependent (CDP) IIF staining pattern either pleomorphic nuclear/nucleolar (PNN, Fig 1 and Fig 2a) or pseudo PCNA (p-PCNA, Fig 2b), but non-reactive with recombinant PCNA (rPCNA).

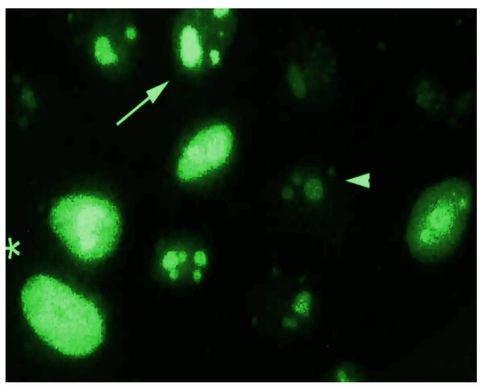

Figure 1 Indirect immunofluorescence pattern of the prototype patient serum (picture taken from Savino et al., 1999, *Journal of Cell Science* 112, 1889-1900).

Methods: The IIF staining pattern was studied on HEp-2 cells (ImmunoConcepts, US; INOVA, US) using monoclonal and/or polyclonal antibodies to PCNA, CENP-F and Nop52 (Santa Cruz Biologicals, US). Western immunoblotting (WB) utilized commercial cell line extract (HEp-2) blots (Trinity Biotech, Ireland; AID, Germany), His-tagged recombinant Nop52 (rNop52) generated from full length human cDNA (Open Biosystems) and expressed in the Gateway Expression system (Invitrogen). Control sera included normal human serum, human sera with previously documented anti-PCNA activity and sera that showed a pattern resembling PCNA but did not react with rPCNA.

Results: WB on cellular extracts showed that these sera reacted with a number of proteins but of most interest were proteins with molecular masses around 50 - 60 kDa. Further analysis of the sera showed that they did not react with rPCNA, Ro52 or Ro60. 11/15 sera with the CDP of interest reacted with rNop52 in WB.

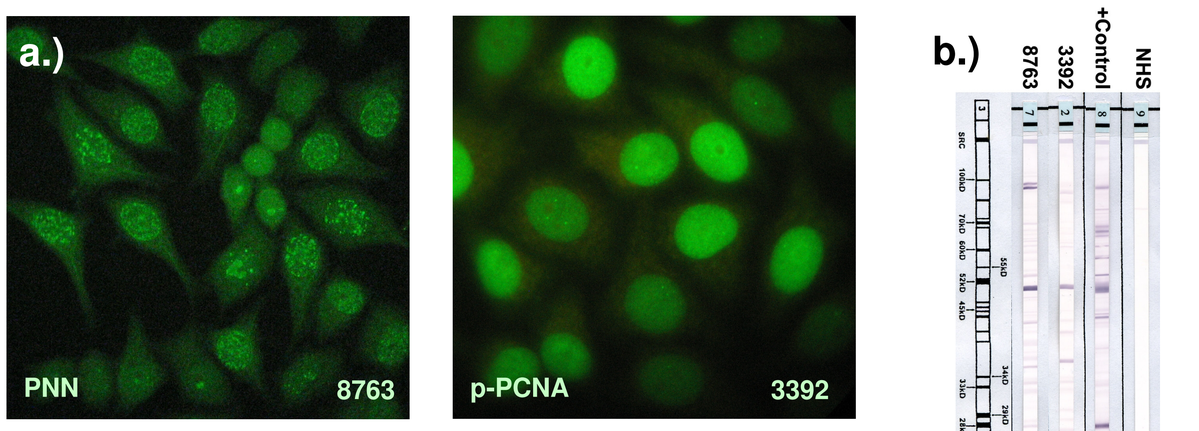

Figure 2 Cell-cycle dependent pleomorphic nuclear/ nucleolar (PNN) and pseudo-PCNA (p-PCNA) pattern is shown in a.). Fig. b.) shows immunoblot results with HEp-2 cell extract.

By contrast, 0/6 anti-PCNA positive sera and none of the normal human sera reacted (Table 1). Clinical features or diagnoses of patients with these aabs included Raynaud's phenomenon, SLE, and undifferentiated connective tissue disease.

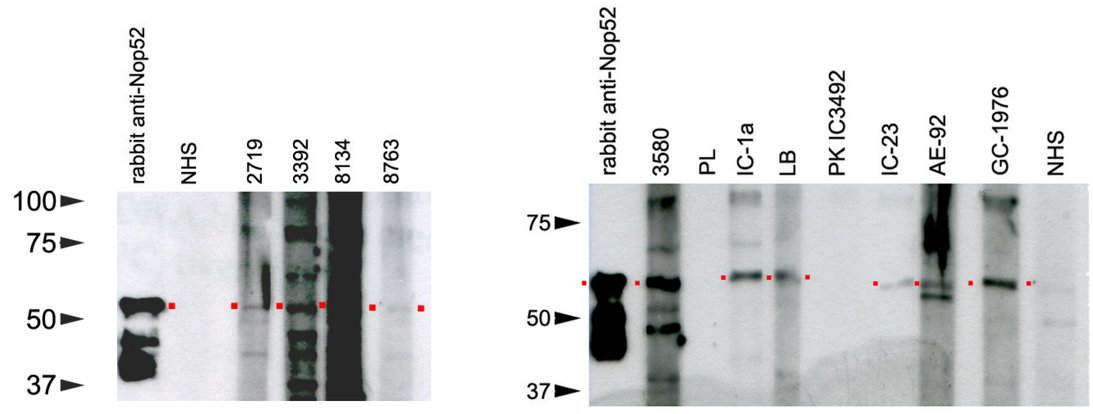

Figure 3 Immunoblot results of two blots with recombinant Nop52. Immunoreactive band of 8134 and YYC-GH was clearly visible on original film. Bands corresponding to Nop52 are marked with red dots.

Table 1 Reactivity profile of samples analysed.

Serum ID	WB Nop52	IIF Pattern	rPCNA	CCP	Clinical	Comments
2719	++	PNN	neg	neg	RA	ENA neg
8763 *	+	PNN	neg	neg	RA	Weak topo-I
8134	++ #	PNN	neg	neg	ISSc	ENA neg
3392	++++	p-PCNA	neg	neg	Atypical SLE, sec. hperparathyreodism	ENA neg
3580	++++	p-PCNA	neg	n.d.	n.d.	n.d.
IC-1a	+++	p-PCNA	neg	n.d.	UCTD	ENA neg
LB	++	p-PCNA	neg	n.d.	SLE	ENA neg
IC-23	+	p-PCNA	neg	n.d.	Raynaud's	ENA neg
AE-92	++	p-PCNA	neg	n.d.	SLE, Alopecia	ENA neg
GC-1976	+++	p-PCNA	neg	n.d.	Raynaud's, RA	RF pos
YYC-GH	++ #	p-PCNA	neg	n.d.	Atypical SLE, Raynaud's	ENA neg
IC3586	neg	p-PCNA	neg	n.d.	SLE	n.d.
LP	neg	p-PCNA	neg	n.d.	Arthtritis	n.d.
PK IC3492	neg	p-PCNA	neg	n.d.	n.d.	n.d.
IC-1135	neg	p-PCNA	neg	n.d.	SLE	n.d.
LB	neg	PCNA	pos	n.d.	SLE	ENA neg
OE	neg	PCNA	pos	n.d.	SLE	ENA neg
PP	neg	PCNA	pos	n.d.	SLE	ENA neg
JB-PCNA	neg	PCNA	pos	n.d.	SLE	ENA neg
AK-PCNA	neg	PCNA	pos	n.d.	SLE	ENA neg
IC-m70	neg	PCNA	pos	n.d.	UCTD	ENA neg
NHS	neg	neg	neg	n.d.	healthy	ENA neg

dark background; p-PCNA = pseudo PCNA; PNN = pleomorphic nuclear/nucleolar; * same patient as 2719

Conclusion: Our study indicates that Nop-52 is a candidate autoantigen and that anti-Nop-52 aabs can be found in sera from rheumatic disease patients with either a PNN or a p-PCNA cell-cycle dependent staining pattern in IIF. Additional clinical and molecular biological studies are underway.