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The many faces of NELL1 MN

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ABSTRACT

Neural tissue encoding protein with EGF-like repeats (NELL1) is a recently discovered target antigen in membranous nephropathy (MN). The initial study showed that most cases of NELL1 MN had no underlying disease associations, i.e. most cases of NELL1 MN were classified as primary MN. Subsequently, NELL1 MN has been found in the setting of various diseases. These include NELL1 MN associated with malignancy, drugs, infections, autoimmune disease, hematopoietic stem cell transplant, de novo MN in a kidney transplant and sarcoidosis. Thus there is marked heterogeneity in the diseases associated with NELL1 MN. Evaluation of an underlying disease associated with MN will likely need to be more exhaustive in NELL1 MN.

Keywords: membranous nephropathy, NELL1

Membranous nephropathy (MN) is characterized by the accumulation of immune complexes in the subepithelial region of the glomerular basement membrane (GBM) [1–3]. The subepithelial immune complexes are primarily composed of a target antigen and immunoglobulin G (IgG) directed towards the target antigen. Until recently, M-type phospholipase A2 receptor (PLA2R) and thrombospondin type-1 domain-containing 7A (THSD7A) were the main target antigens identified in the immune complexes [4, 5]. While PLA2R is quite common and accounts for ≈50–70% of all MN, THSD7A is quite rare and accounts for only ≈1–3% of all MN.

MN is conventionally classified as primary MN, where there is no identifiable underlying disease association. These cases are typically PLA2R-positive on kidney biopsy, accounting for ≈70% of all cases of primary MN. Secondary MN may be associated with an autoimmune disease, infection, malignancy, hematopoietic stem cell transplant (HSCT), sarcoidosis etc. [1–3]. Until recently, the target antigen in almost all cases of secondary MN was not known.

Recently, laser microdissection and tandem mass spectrometry (MS/MS) were used to detect novel proteins/target antigens in MN. The starting point was PLA2R-negative MN, as this excluded a large (≈50–70%) number of MN cases. Although many novel proteins were identified by MS/MS, immunohistochemistry (IHC)/confocal microscopy was then used to localize which of the novel proteins were present along the GBM, thus vastly reducing the number of likely target antigens. Finally, western blot analysis was done using patient serum and/or IgG from frozen biopsy eluate to confirm the presence of antibodies to the novel protein, thus establishing the novel protein as a likely target antigen. Using this sequential approach, Sethi *et al.* [6–10, 12] have recently described six new target antigens in MN that include neural tissue encoding protein with EGF-like repeats (NELL1), exostosin 1/exostosin 2 (EXT1/EXT2), semaphorin 3B (SEMA3B), protocadherin 7 (PCDH7), protocadherin FAT1 and neuron-derived neurotropic factor (NDNF). Furthermore, other groups have described at least three additional target antigens that include contactin (CNTN1), neural cell adhesion molecule 1 (NCAM1) and serine protease HTRA1 [11, 13, 14].

It is easy to postulate that just as PLA2R accounts for most cases of primary MN, a major target antigen would be identifiable in each of the secondary causes, including autoimmune disease, malignancy, drugs, infections, HSCT and sarcoidosis. While this is true for a few of the newer antigens in that they were detected in association with specific diseases, it is not true for many of the other new antigens [6]. Thus EXT1/EXT2 appears

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Probability Legend:	Accession Number	Molecular Weight	Ctrl_06	Primary_01	Primary_02	Autoimmune_01	Autoimmune_02	Autoimmune_03	Hepatitis_01	Hepatitis_02	Hepatitis_03	NSAID_01	NSAID_02	NSAID_03	Sarcoidosis_01	Sarcoidosis_02	Tumor_01	HSCT_01	Hypogammaglobulinemia_01
Protein kinase C-binding protein NELL1 OS=Homo sapiens OX=9606 GN=NELL1 PE=1 SV=4	sp|Q92832|NELL1_HUMAN	90 kDa	0	19	39	31	26	30	34	25	18	32	30	10	20	13	76	23	27
Immunoglobulin gamma-1 heavy chain OS=Homo sapiens OX=9606 PE=1 SV=2	sp|P0DOX5|IGG1_HUMAN	49 kDa	19	27	35	30	24	34	37	33	66	40	21	14	25	31	77	22	27
Immunoglobulin heavy constant gamma 2 OS=Homo sapiens OX=9606 GN=IGHG2 PE=1 ...	sp|P01859|IGHG2_HUMAN	36 kDa	12	16	20	17	15	23	31	17	45	23	21	14	15	21	69	10	22
Immunoglobulin heavy constant gamma 3 OS=Homo sapiens OX=9606 GN=IGHG3 PE=1 ...	sp|P01860|IGHG3_HUMAN	41 kDa	15	17	23	18	23	29	35	22	60	26	23	14	16	20	72	16	30
Immunoglobulin heavy constant gamma 4 OS=Homo sapiens OX=9606 GN=IGHG4 PE=1 ...	sp|P01861|IGHG4_HUMAN	36 kDa	7	13	12	5	13	24	17	11	36	14	14	4	15	20	35	9	15
Secretory phospholipase A2 receptor OS=Homo sapiens OX=9606 GN=PLA2R1 PE=1 SV=2	sp|Q13018|PLA2R_HUMAN	169 kDa	1	3	5	5	2	2	5	1	0	4	3	(0)	(0)	(0)	4	1	2

Probability Legend: over 95%; 80% to 94%; 50% to 79%; 20% to 49%; 0% to 19%

Bio View:
2058 Proteins in 1842 Clusters
With 2057 Filtered Out

Figure 1: MS/MS in NELL1 MN. Representative MS/MS with total spectral counts (TSCs) is shown in 16 cases of NELL1 MN: 2 cases of primary NELL1 (primary 01 and 02), 3 cases with autoimmune diseases (autoimmune 01, 02 and 03), 3 cases with hepatitis (hepatitis 1 is a patient with hepatitis C and hepatitis 2 and 3 are patients with hepatitis B), 3 cases with NSAID use (NSAID 01, 02 and 03), 2 cases of sarcoidosis (sarcoidosis 01 and 02), 1 case with urothelial carcinoma (tumor 01), 1 with HSCT (HSCT 01) and 1 with hypogammaglobulinemia treated with intravenous immunoglobulin (hypogammaglobulinemia 01). Also note that the TSC of IgG1 is greater than for IgG4 in all cases. A baseline PLA2R TSC is present in 12 cases. Controls were glomeruli dissected from a pool of six-time 0 allograft needle biopies.

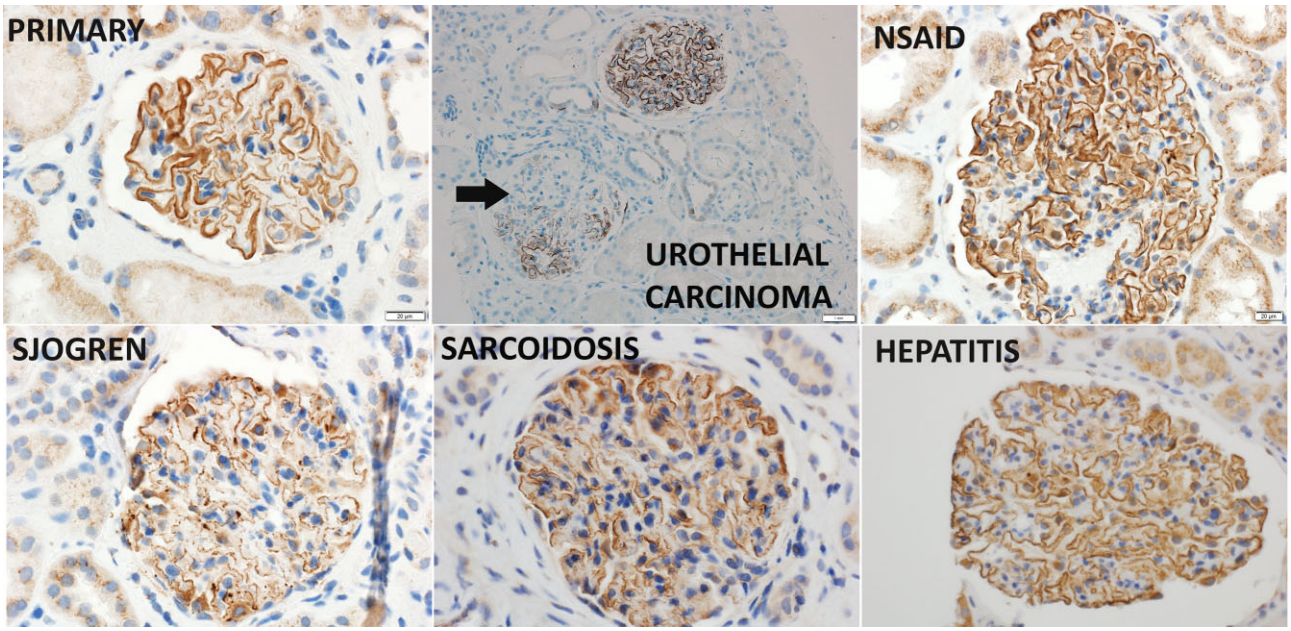

Figure 2: IHC for NELL1. IHC shows granular deposits of NELL1 along the GBM. Note that in the patient with malignancy, there is segmental (arrow) staining for NELL1 in one glomerulus.

to be present almost exclusively in patients with autoimmune disease such as lupus, FAT1 in patients with HSCT and CNTN1 in patients with demyelinating polyneuropathy syndromes. On the other hand, many of the other new target antigens do not fit into a specific secondary disease entity yet have unique characteristics of their own. For example, semaphorin 3B MN is most often detected in the pediatric age group, PCDH7 MN is found in older patients with minimal or no complement activation and NDNF MN appears to have unique kidney biopsy findings where the deposits appear as subepithelial humps with minimal GBM reaction.

The antigen that appears to most defy the conventional classification of primary MN versus secondary MN is NELL1. NELL1 is the second most common target antigen after PLA2R and appears to account for ≈5–10% of all MN cases, after exclusion of autoimmune (lupus) MN. NELL1 has been identified in both primary MN and most diseases associated with MN.

NELL1-MN is everywhere! Figs. 1 and 2 show representative MS/MS and IHC of NELL1 MN, respectively.

1. **NELL1 in primary MN:** Most cases of NELL1 MN do not have an underlying associated disease [8, 15, 16]. In these cases, the kidney biopsy does not show any secondary features (although IgG1 might be the dominant IgG subtype), but importantly, there is no underlying disease association. In the first series of 34 cases of NELL1 MN, 85% of cases did not have an underlying disease association and were labelled as primary MN. In another series of 15 NELL1 MN patients in a Chinese cohort, no secondary disease was found in any of the NELL1 MN patients [16].
2. **NELL1 MN associated with malignancy:** Malignancy is the most common reported disease in secondary NELL1 MN. The incidence of malignancy in NELL1 MN is variable. The highest incidence reported is 33% of NELL1 MN patients had a malignancy compared with 4.2% of PLA2R MN and 10.8% of THSD7A MN, making NELL1 MN the most common type of MN with a malignancy association [17]. Other studies have also found an association of NELL1 MN with malignancy, although it is not as high as 33%. The original discovery study of

NELL1 MN found ≈15% of NELL1 MN patients had an underlying malignancy, and in another smaller study, 20% of NELL1 MN patients had a malignancy (1 of 5 patients) [8, 18]. In contrast, none of the NELL1 MN patients had a malignancy in the Chinese or Japanese patient cohorts [15, 16]. Thus it is reasonable to suggest that the association of NELL1 MN with malignancy may vary based on demographics.

3. **NELL1 MN associated with drugs:** Recently, four patients using lipoic acid were found to have NELL1 MN and discontinuation of lipoic acid resulted in the remission of proteinuria [19]. Drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) have also been reported in a patient with NELL1 MN [15]. In our unpublished series of PLA2R-negative MN, we have detected four cases of NELL1 MN in which patients had a long history of NSAID use (Fig. 1 shows MS/MS data in two of the patients). Traditional indigenous medicines are also associated with NELL1 MN. A recent study found NELL1 positivity in 87.9% of MN associated with traditional indigenous medicines, many of which contained high amounts of mercury. In comparison, only 9.1% of MN associated with indigenous medicines were positive for PLA2R [20].
4. **NELL1 MN associated with autoimmune diseases:** In a short series of four NELL1 MN patients, two of the patients had rheumatoid arthritis [15]. In our own unpublished series of PLA2R-negative MN, we have detected NELL1 MN in patients with cutaneous lupus and Sjögren's syndrome, Hashimoto's thyroiditis, MN with GBM, mesangial and TBM deposits and tubuloreticular inclusions, findings typically seen in patients with autoimmune disease (Fig. 1). We also detected NELL1 MN in a patient who had hypogammaglobulinemia and was treated with intravenous immunoglobulin.
5. **NELL1 MN associated with a transplant:** NELL1 MN has been reported in transplants, both as a de novo MN in kidney transplant and as de novo MN following hematopoietic stem cell transplantation (HSCT) [21, 22]. We have also detected NELL1 in one patient following HSCT for acute myelogenous leukemia (Fig. 1), although FAT1 is the target antigen in >90% of cases of MN following HSCT.
6. **NELL1 MN in pediatric patients:** In general, NELL1 MN occurs in the older population. The mean age of NELL1 MN in two large series was 63.1 and 66.8 years [8, 17]. In a study of pediatric MN patients, one case of NELL1 MN was reported in the pediatric age group [23].

Lastly, we have recently studied MN associated with hepatitis B and sarcoidosis, using MS/MS to detect novel antigens in these settings. Target antigens in hepatitis B and sarcoidosis are largely unknown. To our surprise, we detected NELL1 MN in a subset of patients with either hepatitis B or sarcoidosis.

7. **NELL1 MN associated with hepatitis:** NELL1 MN was detected in two of eight cases (25%) of MN associated with hepatitis B (Fig. 1). In addition, in our unpublished series of PLA2R-negative MN we detected one case of NELL1 MN in a patient with active hepatitis C and cirrhosis.
8. **NELL1 MN associated with sarcoidosis:** NELL1 MN was detected in 4 of 19 cases (21%) of MN associated with sarcoidosis (ASN 2022, FR-PO671) (Fig. 1).

Taken together, NELL1 MN in now reported to be associated with almost all diseases of secondary MN, some diseases appear to be more common than others in NELL1 MN. Clearly, larger studies are needed to carefully dissect NELL1 MN cases and evaluate the incidence of various diseases associated with NELL1 MN. An even more intriguing question is how do these various disease entities all result in NELL1 MN? What is the common pathway? Do they act as a trigger for overexpression of NELL1 or is NELL1 a cryptic antigen that is exposed following an inciting event such a drug or infection or the development of an autoimmune disease or malignancy?

CLASSIFICATION CONUNDRUM

The last question is how do you now classify MN? Does one still go with the primary MN versus secondary MN, or does one classify MN based on the antigen detected, or a combination of the two where evaluation of secondary causes is justified for certain MN antigens?

- Primary versus secondary MN was acceptable when we had only two target antigens and most of primary MN were PLA2R-positive and the remaining were grouped together as PLA2R-negative MN.
- The discovery of new target antigens, including NELL1, now makes it possible not only to accurately type the MN, but also to follow antibody titers to manage patients for serologic response with regards to both remission and relapse. Each new antigen-type MN also appears to have specific clinicopathologic and prognostic features.
- The question now is how can we correlate the new antigen MNs with secondary disease associations. In the end, it is likely that as more data emerge about each new antigen, we can likely generate an algorithm on the type of evaluation needed for each type of MN. For example, an EXT1/EXT2 MN would need a thorough evaluation for autoimmune diseases, SEMA3B for likely genetic abnormality, NDNF for syphilis, FAT1 for HSCT-associated MN and NELL1 for malignancy but also for other etiologies including drugs, infections and sarcoidosis.

To summarize, NELL1 appears to be a unique target antigen in MN. While most cases of NELL1 MN likely fall into idiopathic/primary MN, almost all secondary diseases have been described in association with NELL1 (Fig. 3). Thus evaluation of secondary causes of MN will likely need to be more exhaustive in NELL1 MN. It is also likely that treatment of the secondary causes will result in prolonged remission of NELL1 MN compared with PLA2R MN, which is almost always primary/idiopathic.

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DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article.

CONFLICT OF INTEREST STATEMENT

The author declares no conflicts of interest. The manuscript and data have not been published previously in whole or in part.

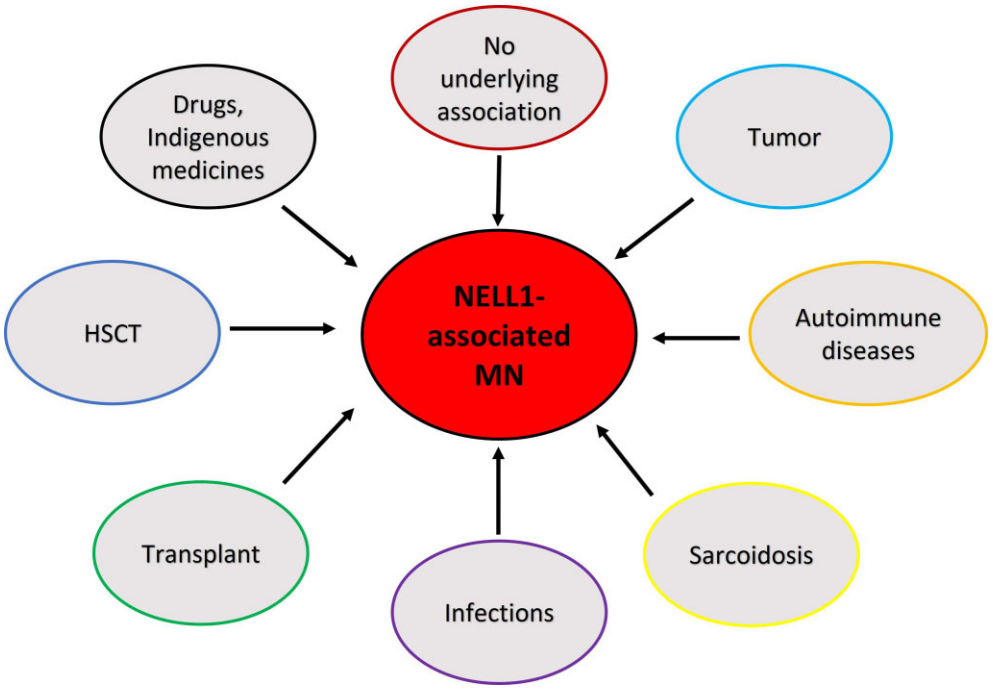

Figure 3: Schematic of various diseases associated with NELL1 MN.

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