Expression and regulatory effects on cancer cell behavior of NELL1 and NELL2 in human renal cell carcinoma

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Kidney cancers, including renal cell carcinoma (RCC) and urothelial carcinoma of the renal pelvis and ureter, account for approximately 3% of all cancer incidences. Although mortality rates of kidney cancers are stabilizing, the number of new cases is increasing in the USA.1–3 Kidney cancers are classified according to their histological type and origin. Cancers originating from kidney epithelia are called RCC. The most common subtype is clear cell RCC (CCRCC), which derives its histological appearance from intracellular accumulations of glycogen and lipid. The risk factors and molecular mechanisms of renal carcinogenesis have been extensively studied. Loss of heterozygosity or mutations of the von Hippel-Lindau (VHL) tumor suppressor gene and the mesenchymal–epithelial transition (c-MET) proto-oncogene, which are responsible for inherited tumor syndromes involving the kidney, have been found in sporadic RCC.4,5 Epigenetic inactivation by DNA methylation was shown to cause downregulation of the mRNA expression of RAS-associated domain family-1A (RASSF1A), a tumor suppressor gene, in RCC.6,7 However, the mechanisms of RCC development and progression remain unclear.

Neural epidermal growth factor-like (Nel) 1 and 2 constitute a family of multimeric and multimodular extracellular glycoproteins. Although the osteogenic effects of NELL1 and functions of NELL2 in neural development have been reported, their expression and functions in cancer are largely unknown. In this study, we examined expression of NELL1 and NELL2 in renal cell carcinoma (RCC) using clinical specimens and cell lines. We show that, whereas NELL1 and NELL2 proteins are strongly expressed in renal tubules in non-cancerous areas of RCC specimens, their expression is significantly downregulated in cancerous areas. Silencing of NELL1 and NELL2 mRNA expression was also detected in RCC cell lines. Analysis of NELL1/2 promoter methylation status indicated that the CpG islands in the NELL1 and NELL2 genes are hypermethylated in RCC cell lines. NELL1 and NELL2 bind to RCC cells, suggesting that these cells express a receptor for NELL1 and NELL2 that can transduce signals. Furthermore, we found that both NELL1 and NELL2 inhibit RCC cell migration, and NELL1 further inhibits RCC cell adhesion. These results suggest that silencing of NELL gene expression by promoter hypermethylation plays roles in RCC progression by affecting cancer cell behavior.
about the expression and functions of NELL1 and NELL2 in RCC. Here we show that, whereas NELL1 and NELL2 are strongly expressed in non-cancerous renal tubules, their expression is downregulated in cancerous areas of CCRCC specimens and in RCC cell lines. In addition, the CpG islands in the NELL1 and NELL2 promoter regions are hypermethylated in RCC cells. In vitro, NELL1 suppresses the migration and adhesion of RCC cells, and NELL2 suppresses RCC cell migration. These results suggest that NELL protein expression is downregulated in RCC, presumably by promoter hypermethylation, and that lack of NELL expression may contribute to RCC progression by altered regulation of cancer cell behavior.

Materials and Methods

Clinical samples. Formalin-fixed paraffin-embedded CCRCC tissues were obtained from surgical pathology files (between 2010 and 2012) of the Pathology Section of Kanazawa University Hospital (Kanazawa, Japan). This project was approved by the research ethics committee on genetic analysis of Kanazawa University (approval no. 320).

Immunohistochemistry. Four-micron sections of CCRCC samples were analyzed by immunohistochemistry (IHC) using anti-NELL1 and anti-NELL2 antibodies (HPA051535, 1/100 and HPA035715, 1/300 dilutions; Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer’s protocol, except that antigen retrieval was carried out at 121°C for 15 min. The LSAB2 kit Universal (Dako, Glostrup, Denmark) was used for detection. NELL1 and NELL2 expression was scored for both cancerous and non-cancerous areas on a graded 0 (no expression) to +3 (very strong expression) scale. Immunohistochemistry scores were calculated by subtracting the scores of cancerous areas from those of adjacent non-cancerous areas. Therefore, positive IHC scores indicated downregulation of NELL expression in cancer cells, whereas negative scores indicated upregulation.

Cell lines. Renal cell carcinoma (OS-RC-2, TUHR14TKB, and VMRC-RCW) and HEK293T cell lines were obtained from the Riken Cell Bank (Tsukuba, Japan). Renal cell carcinoma and HEK293T cells were cultured in RPMI and DMEM, respectively, containing 10% FBS and penicillin/streptomycin.

Quantitative RT-PCR, methylation-specific PCR, and bisulfide sequencing PCR. Quantitative RT-PCR (qPCR) was carried out with first-strand cDNAs of the cell lines and SYBR PremixEx TaqII (Takara Bio, Otsu, Japan) according to the manufacturer’s protocol, except that cDNA synthesis and qPCR were carried out as described above.

Bisulfide sequencing PCR (BSP) was carried out using the bisulfide-treated genomic DNAs and EpiTaq HS (Takara). The PCR products were inserted into the pGEM-T Easy vector (Promega KK, Tokyo, Japan) and transformed into DH5α, and plasmids isolated from 10 colonies of each sample were sequenced. The primer sequences and PCR conditions used are shown in Table 1.

Luciferase assay. NELL1 and NELL2 putative promoter regions were ligated into pCpGL-basic(21) (kindly provided by Prof. Michael Rehli, University Hospital Regensburg, Regensburg, Germany), NELL1 and NELL2 transcriptional factors, RUNX2(22) and E2F1(23) were kindly provided by Prof. Yoshikai Ito (National University of Singapore, Singapore) (pEF-BOS-RUNX2)(24) and Prof. Kristian Helin (University of Copenhagen, BRIC, Copenhagen, Denmark), E2F1/pCMV-HA(25) (Addgene plasmid #24225). RUNX2 was amplified from pEF-BOS-RUNX2 and inserted into pCMV-HA-N (Takara Bio). After incubation with/without methyltransferases, NELL1/pCpGL and NELL2/pCpGL were transfected with their transcriptional factors and pG4.74 into HEK293T cells. The luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega KK).

Preparation of alkaline phosphatase fusion proteins. NELL1 protein fused to an alkaline phosphatase (AP) tag (NELL1-AP), NELL2-AP, and AP proteins were prepared and purified as previously described(26).

Cell migration assay. The cell migration assay was carried out basically as previously described.27 The lower side of the Transwell membrane inserts (product #3422; Corning, Corning, NY, USA) was coated with 40 nM or 400 nM AP, NELL1-AP, or NELL2-AP, and AP proteins were prepared and purified as previously described.26

Table 1. Primer sequences and PCR conditions

| Gene  | PCR                  | Forward primer sequence (5’–3’)               | Reverse primer sequence (5’–3’)               | Annealing temperature | PCR cycles |
|-------|----------------------|------------------------------------------------|------------------------------------------------|-----------------------|------------|
| NELL1 | Quantitative RT-PCR  | ATGGCCAGGTGGTGGACCTTG                        | TCTGGTGTCAATTTCTGGG                           | 60                    | 40         |
| NELL2 | Quantitative RT-PCR  | CCACTAACATGCGCTTTCG                          | ATGGTCTGTCGTCCTGGCACT                       | 60                    | 40         |
| GAPDH | Quantitative RT-PCR  | GTCTCCTGCTACCTAAAAGGGCG                      | ACCACCTGTTGTGCTAGCC                          | 60                    | 40         |
| NELL1 | MSP-Methyl.          | GATTCCGATTTCCTGGGTTTC                       | CTATGGTGTCAATTTCTGGG                         | 40                    | 35         |
| NELL2 | MSP-Methyl.          | CTGTATTTTATGTAGGGG                          | TTCCACCCGGAAAAAAGAA                        | 60                    | 35         |
| NELL1 | MSP-Unmethyl.        | TGGGATTTTATGTAGGGG                          | ACTCATTACTCAAAAAAAAACCAAC                   | 55                    | 40         |
| NELL2 | MSP-Unmethyl.        | TTATGTTTTTATGTAGGGG                         | AATTTCCACAAAAAAAACAC                      | 63                    | 35         |
| NELL1 | Bisulfite sequencing | TATAGGGGTTAATAGGAGAGAGAG                    | CCAATCTAAGACCC*CCCACTACTAAA                 | 55                    | 35         |
| NELL2 | Bisulfite sequencing | TGTTTTGTGTATGTAGGGG                         | AAAAAGGAACCTCCCCAAGA                     | 55                    | 35         |

* R(A=G). Methyl., methylated; MSP, methylation-specific PCR; Unmethyl., unmethylated.
high-power fields were examined under a microscope. Each experiment was repeated three times.

**Cell adhesion assay.** Ninety-six-well cell culture plates were coated with AP, NELL1-AP, or NELL2-AP protein (400 nM each) at 4°C overnight. After blocking with 10 mg/mL BSA, the wells were washed twice with PBS, and OS-RC-2 or VMRC-RCW cells were then plated at a density of 2.5 × 10^4 cells/well. The cells were cultured at 37°C for 3 h in RPMI-1640 with 2% FBS, washed twice with PBS, and then fixed with 4% paraformaldehyde. Cell numbers in three high-power fields per well were counted using an inverted microscope. Experiments were repeated under the same conditions at least three times.

**Statistical analysis.** For statistical analysis, ANOVA (for IHC scores with more than three parameters) and Student’s t-test (for IHC scores with two parameters, and cell migration and adhesion assays) were used. Supplementary information is available in Document S1.

**Results**

**NELL1 and NELL2 protein expression is downregulated in CCRCC.** We first examined and compared NELL1 and NELL2 protein expression in cancerous regions and adjacent non-cancerous areas in tissue samples of 64 CCRCC patients. Both NELL1 and NELL2 proteins were abundantly expressed in renal tubules in non-cancerous regions (Fig. 1). At higher magnification (Fig. 1e,g), NELL1 and NELL2 proteins were observed to be localized in granular cytoplasmic patterns, consistent with the fact that NELL1 and NELL2 are secreted glycoproteins and thus processed in the secretory pathway. In contrast, NELL1 and NELL2 expression was significantly lower in cancerous areas (IHC scores, 1.88 (NELL1) and 2.17 (NELL2); expression grade of cancerous area/non-cancerous area, 1.05/2.09 (NELL1, *P* < 0.01) and 0.72/2.93 (NELL2, *P* < 0.01); Fig. 1h). No significant correlations were observed between the degree of NELL downregulation and clinicopathological parameters, including sex or age of patients, or vascular involvement or grade of tumors (Table 2).

**Downregulation of NELL1 and NELL2 expression in RCC cell lines.** We next examined and compared NELL1 and NELL2 mRNA and protein expression in RCC cell lines with that in the HEK293T cell line derived from human embryonic kidney using qRT-PCR and Western blot. We found by RT-PCR that NELL1 and NELL2 mRNA expression levels in the cancer cell lines were 10–1000 times lower than those in HEK293T cells.
islands in these regions in the 5′oter regions of
ried out BSP of the CpG islands that included the putative pro-
(Fig. 3). No significant methylation was detected in the
RCW cells, and weak hypermethylation was observed in OS-
was also significantly increased after 5-azacytidine treat-
alyses of the NELL1 promoter region showed methylation of
CpG islands around No. 10 in both the OS-RC2 and
VMRC-RCW cells and around No. 80 in the OS-RC-2 cells
(Fig. 4d). No significant CpG island hypermethylation was
detected at the NELL1/2 promoter regions in the HEK293T
cells.
If the NELL1/2 downregulation observed in RCC cells
is caused by promoter hypermethylation, the expression would
be recovered by CpG demethylation. In order to test this hypo-
thesis, we treated RCC cell lines with 5 μM 5-azacytidine and
examined the effects on the NELL1/2 mRNA expression. As
shown in Figure 4(e), the NELL1 expression was elevated in
VMRC-RCW cells. The NELL1 expression in TUHR14TKB
cells was also significantly increased after 5-azacytidine treat-
ment. The NELL2 expression in OS-RC-2 cells was elevated
by 5-azacytidine treatment (Fig. 4f). A slight increase in
NELL2 expression was observed in VMRC-RCW cells,
although the difference was not statistically significant
(P = 0.071). Whereas the increases of NELL1 and NELL2
expression by 5-azacytidine were statistically significant
in most of the above RCC cell lines, their expression levels
after treatment were still lower than those in HKE293T
(Figs 2,4e,f), suggesting that promoter methylation partially
contributes to the silencing of NELL expression.
In order to confirm the effects of CpG methylation on
NELL1/2 expression, we carried out a luciferase assay using
the putative promoter regions of NELL1 and NELL2. Conse-
sequently, reporter constructs containing unmethylated NELL1
and NELL2 promoter regions showed high luciferase activity.
In contrast, the luciferase activity was significantly reduced
by treatment with M.SssI, which methylates all cytosine residues
within the dinucleotide recognition sequence 5′-CpG-3′. Treat-
ment with HpaII, which methylates the second cytosine residue
in 5′-CCGG-3′, or HhaI, which methylates the first cytosine
residue in 5′-GCGC-3′, resulted in partial reduction of the
luciferase activity (Fig. 4g,h).

Table 2. Correlation between neural epidermal growth factor-like like 1 (NELL1) and NELL2 subtraction scores (immunohistochemistry [IHC] score) and clinicopathological parameters

| No. of cases | Average of NELL1 IHC scores | P-value | Average of NELL2 IHC scores | P-value |
|--------------|-----------------------------|---------|-----------------------------|---------|
| Overall      | 64                          | 1.88    | 2.17                        |         |
| Sex          |                             |         |                             |         |
| Male         | 43                          | 1.84    | 0.71†                       | 2.16    | 0.93† |
| Female       | 21                          | 1.95    |                             | 2.19    |        |
| Age, years   |                             |         |                             |         |        |
| <60          | 26                          | 1.65    | 0.19†                       | 2.30    | 0.46‡ |
| ≥60          | 38                          | 2.03    |                             | 2.37    |        |
| Grade        |                             |         |                             |         |        |
| G1           | 20                          | 2.10    | 0.18‡                       | 2.30    | 0.46‡ |
| G2           | 43                          | 1.74    | 0.86†                       | 2.19    | 0.86† |
| G3           | 1                           | 3.00    |                             | 2.14    |        |
| Vascular involvement |               |         |                             |         |        |
| v0           | 42                          | 1.86    | 0.85§                       | 2.11    | 0.69§ |
| v1           | 22                          | 1.90    |                             | 2.14    |        |
| pT           |                             |         |                             |         |        |
| pT1          | 47                          | 1.83    |                             | 2.11    | 0.69§ |
| pT2          | 4                           | 2.00    |                             | 2.50    |        |
| pT3          | 13                          | 2.00    |                             | 3.11    |        |
| pT4          | 0                           |         |                             |         |        |

†Student’s t-test was carried out. ‡More than three parameters, however only two parameters had more than three samples. Student’s t-test was carried out between G1 and G2. §ANOVA was carried out.
RCC was caused, at least in part, by promoter hypermethylation. We previously showed that downregulation of NELL1 promoter regions is hypermethylated in RCC, and suggested that downregulation of NELL1 and NELL2 expression may suggest that downregulation of NELL1 and NELL2 expression in renal cell carcinoma cells. Genomic DNA was analyzed by PCR using specific primers for methylated (Methyl) or unmethylated (Unmethyl) CpG islands in NELL1 or NELL2 putative promoter regions (NELL1 methylated, NELL1 unmethylated, NELL2 methylated, and NELL2 unmethylated).

These data indicate that the CpG island of NELL1 and NELL2 promoter regions is hypermethylated in RCC, and suggest that downregulation of NELL1 and NELL2 expression in RCC was caused, at least in part, by promoter hypermethylation.

NELL1 and NELL2 bind to RCC cells. The above data on NELL1 and NELL2 expression may suggest that downregulation of NELL1 and NELL2 gene expression is involved in the development of RCC. If that is the case, it is likely that NELL1 and NELL2 proteins exert their functions by binding to a cell surface receptor and transducing intracellular signals. We therefore examined whether NELL proteins can bind to RCC cell lines using NELL1-AP or NELL2-AP as a probe (Fig. S2a). We incubated RCC and HEK293T cells with NELL1-AP or NELL2-AP, and binding was detected using the AP enzyme reaction. Significant binding activity was detected in all cell lines tested, including HEK293T cells (Fig. S2b). These results indicate that NELL1 and NELL2 can bind to RCC cells, and suggest that NELL1 and NELL2 can be recognized through a specific receptor expressed on those cells.

Effects of NELL proteins on RCC cell behavior. We previously showed that chicken Nel (NELL2) can act as an inhibitory axon guidance molecule and regulate morphology of the growth cone. As many axon guidance molecules also regulate behavior of the cell body, we examined whether NELL proteins can affect the migration and adhesion of RCC cells. We first tested the effects of NELL1 and NELL2 on cell migration by using a transfilter assay system. NELL1-AP significantly inhibited the migration of OS-RC-2 and VMRC-RCW cells in a dose-dependent manner. NELL2-AP also significantly inhibited the migration of OS-RC-2, but not that of VMRC-RCW cells (Fig. 5a–d).

We next examined whether NELL1 or NELL2 proteins affect cell adhesion using NELL-AP protein-coated dishes. Significant numbers of OS-RC-2 and VMRC-RCW cells adhered to the AP-coated control dish. In contrast, the number of adherent cells on the NELL1-AP substratum was dramatically decreased. NELL2-AP did not affect adhesion of the RCC cells (Fig. 5e–i). The combined results indicate that NELL1 and NELL2 can regulate RCC cell behavior.

Discussion

In the present study, we analyzed the expression, genomic DNA methylation, and functions of NELL1 and NELL2 in RCC. Immunohistochemical studies of clinical samples showed that, whereas NELL1 and NELL2 are strongly expressed in non-cancerous renal tubules, their expression is significantly downregulated in CCRCC areas (Fig. 1). Consistent with the results of clinical samples, NELL1 and NELL2 mRNA and protein expression was also reduced in RCC cell lines compared with that in HEK293T cells, which are derived from non-cancerous kidney (Figs 2.S1).

NELL1 and NELL2 expression has been previously reported in other cancers using clinical samples and cell lines. For example, NELL1 gene loss was observed in more than 40% of Hodgkin’s lymphoma patients. NELL2 mRNA expression is upregulated in benign prostate hyperplasia and prostate cancer. NELL2 is also considered as a candidate biomarker for bladder cancer. Among nervous system tumors, NELL1 and NELL2 are predominantly expressed in neuroblastoma cell lines and are also expressed in medulloblastoma, central neurocytoma, and some astrocytic tumors. A recent study showed that posterior fossa ependymoma is composed of two molecularly distinct groups, and that NELL2 is the most significant marker for the group that represents less invasive and less metastatic tumors with better prognosis. These results suggest that NELL may be involved in cancer development.

Our studies of DNA methylation status showed that the CpG islands in the NELL1 and NELL2 promoters are hypermethylated in RCC cells, whereas no significant NELL1/2 promoter hypermethylation was detected in control HEK293 cells. The
Fig. 4. Hypermethylation of CpG islands in the putative promoter region of the NELL1 and NELL2 genes. (a, c) CpG islands (vertical bars) in the putative promoter regions of NELL1 (a) and NELL2 (c) genes are numbered in the 5’ to 3’ direction (NELL1, Nos. 1–78; NELL2, Nos. 1–86). Positions of primer sequences for methylation-specific PCR (MSP) and bisulfide sequencing PCR (BSP) are also shown. (b, d) Methylation status of CpG islands. ●, Methylated CpG islands (methylation detected in more than 8 of 10 clones); ○, unmethylated islands (methylation not detected in any of 10 clones); △, incompletely methylated CpG islands (methylation detected in one to seven clones). * This nucleotide in HEK293T differed from the registered sequence in the database. (e, f) NELL1 and NELL2 mRNA expression levels in OS-RC-2, VMRC-RCW and TUHR14TKB cells with/without 5-azacytidine treatment. The NELL1, NELL2 and GAPDH mRNA expression levels were measured using quantitative PCR. NELL1 and NELL2 mRNA expression levels were normalized by that of GAPDH and plotted as the mean ± SD. (g, h) Effects of promoter methylation on NELL1 and NELL2 expression. Firefly luciferase reporter constructs containing putative promoter regions of NELL1 and NELL2 genes (NELL1/pCpGL and NELL2/pCpGL) were treated with M.SssI, HpaII, or HhaI methyltransferases and then transfected into HEK293T cells with RUNX2/pCMVHA (for NELL1/pCpGL) or E2F1/pCMVHA (for NELL2/pCpGL) and the Renilla luciferase expression vector pGL 4.74. The ratio of the firefly/Renilla luciferase activity was plotted as the mean ± SD.
degree of promoter hypermethylation and effect of 5-azacytidine treatment varied between RCC cell lines. In the RCC cell lines in which promoter hypermethylation was particularly prominent (NELL1 in VMRC-RCW and TUHR14TKB cells, NELL2 in OS-RC-2 cells), the NELL mRNA expression was significantly recovered by 5-azacytidine treatment (Fig. 4e,f). In contrast, recovery of NELL2 expression was not statistically significant in VMRC-RCW and TUHR14TKB cells. Interestingly, CpG islands around No. 80 in the NELL2 promoter were methylated only in OS-RC-2, but not in VMRC-RCW or TUHR14TKB cells (Fig. 4d). In addition, NELL2 expression levels without treatment were much lower in OS-RC-2 cells than those in VMRC-RCW or TUHR14TKB cells. These results suggest that CpG methylation around No. 80 may be important for NELL2 downregulation, although it is plausible that other factors are also involved in NELL2 downregulation in OS-RC-2 cells, because the NELL2 expression level restored by 5-azacytidine treatment was still lower than those in untreated VMRC-RCW, TUHR14TKB, and HEK293T cells.

DNA methylation of gene promoters is an important mechanism of transcriptional downregulation of gene expression. In cancer cells, many tumor suppressor genes have been shown to be silenced by promoter hypermethylation, including p16/CDKN2/MTS1, retinoblastoma gene, and EphA7. In RCC, transcriptional silencing by promoter hypermethylation was reported for several tumor-suppressor genes, including VHL and RASSF1A and differentially expressed in adenocarcinoma of the lung (DAL-1/4.1B). DNA methylation profiling of 10 tumor suppressor genes in
100 kidney cancers showed hypermethylation of promoter DNA in 93% of kidney tumor cases, approximately two-thirds of which have two or more hypermethylated genes. (38)

Promoter hypermethylation and silencing of the NELL1 gene were detected in approximately 44% of colorectal carcinomas. (29) In esophageal cancer, methylation levels of the NELL1 gene are significantly higher in Barrett’s metaplasia, dysplasia in Barrett’s esophagus, and esophageal adenocarcinoma than those in normal esophagus, and inversely correlate with patient survival rates. These findings indicate that promoter hypermethylation of NELL1 is a common event that occurs at early stages of Barrett’s-associated esophageal cancer progression and is a potential biomarker of poor prognosis in early stage esophageal adenocarcinoma. (28)

In vitro assays of cell behavior in the present study indicated that both NELL1 and NELL2 inhibit RCC cell migration and that NELL1 further inhibits RCC cell adhesion. As regulation of cell migration and adhesion plays crucial roles in cancer invasion and metastasis, these results raise the possibility that loss of NELL expression in RCC is involved in cancer progression. Although no significant correlation was found between NELL expression levels and the size or extent of the primary tumor (pT stages), our data show that hypermethylation and downregulation of the NELL genes already occurs at early stages of RCC progression. These findings are consistent with esophageal cancer data, (28) and suggest that decreased NELL expression plays roles in early oncogenesis.

Our results showing that NELL1 and NELL2 bind to RCC cells suggest that these molecules regulate the behavior of cancer cells by binding to a cell surface receptor and transducing intracellular signals. We also previously reported that chicken Nel (NELL2) can bind to and regulate the behavior of retinal axons. (15) Although no receptors have been identified for NELL2, recent studies have shown that integrins (β1 (39) and α3β1 (40)) act as cell surface receptors for NELL1. Binding of NELL1 to these integrins promotes adhesion of several different types of cells, including bone marrow stromal cells, multipotential mesenchymal cells, chondroprogenitor cells, pre-osteoblasts, and human osteocarcinoma cells. (39,40) As it is likely that NELL1 protein interacts with a variety of receptors through its different domains, the opposite effects of NELL1 on cell adhesion may be mediated by different NELL1 receptors that interact with different domains of NELL1. Further studies, especially regarding identification of additional receptors and signaling pathways, are required to fully understand NELL functions in cancer cell behavior regulation.

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Disclosure Statement

The authors have no conflict of interest.
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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Neural epidermal growth factor-like like 1 (NELLI) and NELL2 protein expression in renal cell carcinoma cell lines.

Fig. S2. Binding activity of neural epidermal growth factor-like like protein fused to an alkaline phosphatase tag (NELL-AP) to renal cell carcinoma cells.

Doc. S1. Supplementary materials and methods.