Biochemical and Biophysical Research Communications 460 (2015) 368–374

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

NELL-1 expression in benign and malignant bone tumors

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ARTICLE INFO

Article history:
Received 21 February 2015
Available online 17 March 2015

Keywords:
Nell-1
Osteoid osteoma
Osteoblastoma
Osteosarcoma

ABSTRACT

NELL-1 (NEL-like Protein 1) is an osteoinductive protein with increasing usage as a bone graft substitute in preclinical animal models. NELL-1 was first identified to have bone-forming properties by its overexpression in fusing cranial sutures. Since this time, addition of recombinant NELL-1 has been used to successfully induce bone formation in the calvarial, axial and appendicular skeleton. With increasing interest in the use of NELL-1 as a bone-graft substitute, we sought to examine the expression of NELL-1 in a wide spectrum of benign and malignant bone-forming skeletal tumors. Immunohistochemical expression was examined in human pathologic specimens. Quantitative RT-PCR evaluated NELL-1 expression among OS cell lines *in vitro*. Results showed NELL-1 expression in all bone tumors. Likewise, all OS cell lines demonstrated increased *NELL-1* expression in comparison to non-lesional human bone marrow stromal cells. Among, benign bone tumors (osteoid osteoma and osteoblastoma), strong and diffuse staining was observed, which spatially correlated with markers of osteogenic differentiation. In contrast, a relative reduction in NELL-1 staining was observed in osteosarcoma, accompanied by increased variation between tumors. Among osteosarcoma specimens, NELL-1 expression did not correlate well with markers of osteogenic differentiation. Surprisingly, among osteosarcoma subtypes, fibroblastic osteosarcoma demonstrated the highest expression of NELL-1. In summary, NELL-1 demonstrates diffuse and reliable expression in benign but not malignant bone-forming skeletal tumors. Future studies will further define the basic biologic, diagnostic and prognostic importance of NELL-1 in bone neoplasms.

1. Introduction

NELL-1 (NEL-like Protein 1) is an osteoinductive protein with increasing usage as a bone graft substitute in preclinical animal models. NELL-1 was first identified to have bone-forming properties by its overexpression in fusing cranial sutures [1]. Conversely, *Nell-1* deficient mice exhibit cranial and vertebral bone defects with under-mineralization [2]. NELL-1 is a secreted protein of 810-amino acids with a molecular weight of about 90 kDa before N-glycosylation and oligomerization [3]. Mechanistically, NELL-1 has been recently shown to bind the cell surface receptor integrin $\beta1^4$, and regulates activity of the master osteogenic transcription factor, Runt-related transcription factor-2 (Runx2) [5]. In addition to its significant pro-osteogenic effects, NELL-1 has been shown to induce chondroprogenitor cell proliferation [6] and inhibit adipogenic differentiation [7]. Recombinant NELL-1 has been used to successfully induce bone formation in the calvarial, axial and appendicular skeleton across multiple animal models [8–12].

Abbreviations: BMP, Bone Morphogenetic Protein; BSP, Bone Sialoprotein; OCN, Osteocalcin; OS, Osteosarcoma; Nell-1, NEL-like Protein 1; Runx2, Runt-related transcription factor-2.

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http://dx.doi.org/10.1016/j.bbrc.2015.03.040
0006-291X/

The importance of avoiding tumorigenesis cannot be overemphasized in the field of bone tissue engineering and regeneration. This issue has growing importance with protein-based bone repair. For example, the main FDA approved recombinant protein for bone formation is BMP2 (Bone Morphogenetic Protein 2) [13,14]. BMP ligands and BMP receptors are expressed in most osteosarcoma cell lines and osteosarcoma subtypes [15,16]. Moreover, although disagreement in the literature exists, the presence of BMP signaling in osteosarcoma may impart a worse prognosis [16–18]. On the cellular level, BMP signaling appears to mediate pro-migratory effects in both osteosarcoma and chondrosarcoma cell types [19]. Likewise, Parathyroid Hormone is the main FDA approved anabolic agent in the treatment of osteoporosis [20–23]. Unfortunately, the clinical duration of use for PTH is limited to 24 months, owing to the risk of osteosarcomagenesis [24]. Thus, currently approved agents for bone formation are not without potential risks for sarcomagenesis.

Despite increasing interest in the use of NELL-1 based bone graft substitutes for skeletal tissue engineering, there is to date essentially no description of NELL-1 expression in bone tumors. Several pieces of data suggest that NELL-1 expression in down-regulated in epithelial malignancies. For example, in a genome-wide search of molecules with epigenetic silencing in colon cancer, NELL-1 was found to have frequent methylation along with seven other genes [25]. Likewise, NELL-1 has been found to be epigenetically silenced in esophageal adenocarcinoma [26]. Despite this, the expression of NELL-1 in bone-forming skeletal tumors is entirely unknown.

2. Materials and methods

2.1. Antibodies and reagents

Primary antibodies used in this study were anti-NEL like protein 1 (NELL-1) (GTX111493, GeneTex, Inc., Irvine, CA), anti-Osteocalcin (OCN) (sc-30044, Santa Cruz Biotechnology, Santa Cruz, CA), and anti-Bone Sialoprotein (BSP) (AB1854, Millipore, Billerica, MA). All other reagents were purchased from Dako unless otherwise specified.

2.2. Tissue procurement

Tumors were retrospectively collected from biopsy and resection specimens at the University of California, Los Angeles under IRB# 13-897. Each tumor was re-examined by two blinded bone tissue pathologists to ensure accuracy of original diagnosis. Demographic features and specific tumor measurements were recorded, including patient age, gender, anatomic location, tumor size, history of neoadjuvant therapy, length of follow-up, and clinical course including regional recurrence and distant metastasis.

2.3. Histological and immunohistochemical analyses

Five-micron-thick paraffin sections of bone tumors were stained with haematoxylin and eosin (H&E). Using H&E sections, histomorphologic assessments were made to confirm tumor type and to determine characteristics of different regions within each section. Additional sections were analyzed by indirect immunohistochemistry. Briefly, unstained sections were deparaffinized in xylene and a series of graded ethanol solutions, and rehydrated using phosphate buffered solution. The slides were incubated in 3% hydrogen peroxide for 20 min at room temperature to block endogenous peroxidase activity. 0.125% trypsin induced epitope retrieval was performed for 20 min at room temperature, using the "Digest-All 2" system (Cat 00-3008, Invitrogen, Grand Island, NY). Slides were then incubated with the primary antibody for 1 h at 37° Celsius and 4° Celsius overnight. The anti-NELL-1 primary antibody was used at a dilution of 1:400, the anti-Bone Sialoprotein (BSP) primary antibody was used at a 1:2000 dilution, and the anti-Osteocalcin (OCN) primary antibody was used at a 1:200 dilution. After incubation with the primary antibody, slides were incubated with the appropriate biotinylated secondary antibodies (Dako) for 1 h at room temperature. All secondary antibodies were used at a 1:200 dilution.

Positive immunoreactivity was detected following ABC complex (PK-6100, Vectastain Elite ABC Kit, Vector Laboratories Inc., Burlingame, CA) incubation and development with AEC chromagen (K346911-2, Dako). Negative controls for each antibody consisted of incubation with secondary antibody in the absence of primary antibody. Sections of neonatal rat spines were used in each instance as a positive staining control. Sections were counterstained in Modified Mayers Hematoxylin (Thermo Scientific, Waltham, MA) for 30 s, and placed under running water for 5 min. Slides were mounted using aqueous mounting medium (Dako). Photomicrographs were acquired using Olympus BX51 (100× & 200× magnification lens, UPLanFL, Olympus).

Intensity and distribution of immunohistochemical staining were determined by three blinded observers. The intensity of staining was estimated using a 3 point scale, with '0' indicating no staining, '1+' indicating predominantly faint/barely perceptible cytoplasmic staining within any percentage of tumor cells, '2+' indicating predominantly weak/moderate cytoplasmic staining within any percentage of tumor cells, and '3+' indicating strong/intense cytoplasmic staining within any percentage of tumor cells. Discrepancies in semi-quantification of intensity of staining between observers were found in less than 10% of samples. In this case, the intensity of stain was determined by consensus re-review of the slides by all three observers. Distribution of staining was determined on a continuous 0%–100% scale, estimating the percentage of tumor cells with NELL-1 immunoreactivity. Distribution of staining for each tumor was determined as a mean value between each blinded observer. Comparison of NELL-1 immunoreactivity to markers of bone formation (BSP, OCN) was evaluated on a three part descriptive scale, including 'non-concordant,' 'somewhat concordant,' and 'concordant.' Again, in cases of disagreement between blinded observers, a consensus re-review of the slides in question was performed.

2.4. Quantitative RT-PCR

Cells were expanded in standard growth medium (DMEM, 10% FBS), as previously described [4]. Real time PCR for *NELL-1* was performed as previously described, performed in triplicate wells per RNA isolate [4].

2.5. Statistical analysis

Statistical analysis was performed using an appropriate Student's *t*-test when two groups of numerical values were being compared, as in the case of staining distribution. A Fisher's exact test was performed to determine statistical significance of contingency tables, as in the case of staining intensity. In general, a p-value less than 0.05 was considered statistically significant.

3. Results

3.1. NELL-1 expression in benign bone tumors

Non-lesional trabecular bone showed minimal NELL-1 immunoreactivity in any cell type (Fig. 1A and B). Demographic

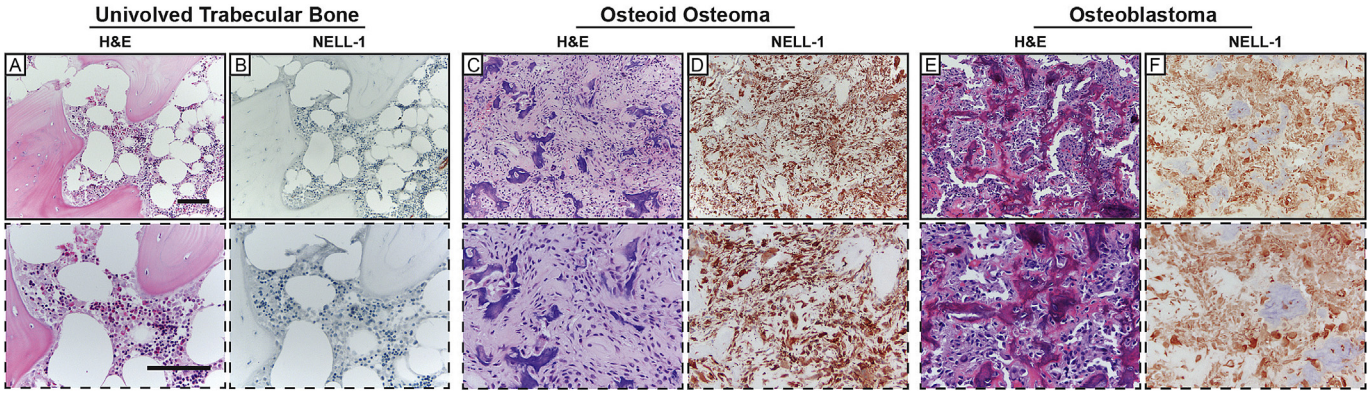

Fig. 1. NELL-1 expression in benign bone forming tumors and non-lesional bone. Representative H&E staining and NELL-1 immunohistochemical staining in (A,B) non-lesional human trabecular bone, (C,D) Osteoid osteoma, and (E,F) Osteoblastoma. Images shown use the GeneTex NELL-1 antibody. Scale bar: 100 μm.

information can be found in Supplementary Table 1. All cases of osteoid osteoma and osteoblastoma demonstrated characteristic anastomosing trabeculae of woven bone, with a single layer of activated osteoblasts and variable multi-nucleated osteoclasts (Fig. 1C–F). Strong and diffuse NELL-1 immunoreactivity was observed in the nidus of all tumors including the majority of bone-lining osteoblasts as well as bone-entombed osteocytes. Immunoreactivity was generally in a cytosolic or combined cytosolic and nuclear distribution. Other cell types within the nidus also demonstrated NELL-1 immunoreactivity, including multinucleated osteoclastic cells and cells embedded within the fibrous stroma. Focal immunostaining of the tumor osteoid matrix was also seen in the minority of cases. Semi-quantification of NELL-1 immunoreactivity in benign tumors demonstrated intermediate to strong intensity of staining in all samples (2-3+) with the majority of tumor cells showing immunoreactivity (on average 76% and 71% of tumors cells within osteoid osteoma and osteoblastoma, respectively) (Tables 1 and 2).

3.2. NELL-1 expression in osteoblastic osteosarcoma

Next, NELL-1 immunoreactivity was examined in biopsy specimens from osteoblastic osteosarcoma. Demographic information can be found in Supplementary Table 2. All biopsy samples were initial diagnostic material, and thus no patients had a history of chemotherapy or radiation. Results showed that among osteoblastic OS biopsy samples a wide variability in NELL-1 expression was observed (Fig. 2). Although some degree of NELL-1 immunoreactivity was observed in all cases, this varied from scattered, focal and weak staining in some cases to strong and diffuse immunoreactivity in other cases. The degree of NELL-1 staining did not appear to correlate with either the degree, thickness or pattern of ossification or calcification within the biopsy material. Resection specimens of osteoblastic osteosarcoma with or without neoadjuvant chemotherapy were next examined. Treatment effect was noted at anywhere from 60% to 90% necrosis in those cases with neoadjuvant therapy (Supplementary Table 2). Semi-quantification of NELL-1 immunohistochemical staining also reflected a wide range in variability among osteoblastic OS samples (Table 1). The intensity of staining ranged from weak to intense (1-3+) with on average approximately 48% (±27%) of tumor cells demonstrating positive staining (n = 8 specimens).

3.3. NELL-1 expression in osteosarcoma subtypes

We next sought to examine NELL-1 expression among OS with different morphologic patterns (including chondroblastic and fibroblastic OS), as well as distinct OS subtypes (including parosteal, periosteal, and telangiectatic OS). The histologic appearance and distribution of NELL-1 immunoreactivity was compared on serial sections of chondroblastic OS (Fig. 3). Chondroblastic OS was seen as a morphologic variant alone or in conjunction with osteoblastic OS. Among chondroblastic OS, a wide variety of NELL-1 immunostaining patterns was seen within a single tumor, often with alternating areas of complete absence and relative abundance of NELL-1 immunoreactivity. Intermediate staining intensity was observed throughout, with the minority of tumor cells stained (31%). Generally, increased immunoreactivity was noted in more hypercellular/higher grade areas of chondroblastic OS. Next, fibroblastic OS specimens were examined. Surprisingly, fibroblastic OS specimens showed increased intensity and distribution of NELL-1 immunostaining in comparison to other morphologic OS variants (2-3+ staining intensity, 68% of tumor cells). As well, NELL-1 immunoreactivity did not seem to correlate well with osteoid production (Fig. 3), but rather was most notable in areas of sheeting of fibroblastic tumor cells.

Table 1
Semi-quantitative assessment of NELL-1 immunohistochemistry, by tumor type.

Tumor type	*Staining intensity (% of cases stained)*				*Staining distribution*
	0	1+	2+	3+	Mean % cells stained (±SD)
Osteoid Osteoma	–	–	–	5/5 (100%)	76% (±13%)
Osteoblastoma	–	–	2/5 (40%)	3/5 (60%)	71% (±6%)
Osteoblastic OS	–	1/8 (13%)	2/8 (25%)	5/8 (63%)	48% (±27%)
Chondroblastic OS	–	–	4/4 (100%)	–	31% (±17%)
Fibroblastic OS	–	–	1/2 (50%)	1/2 (50%)	68% (±22%)
Parosteal OS	–	3/6 (50%)	3/6 (50%)	–	28% (±18%)
Periosteal OS	–	–	1/1 (100%)	–	80%
Telangiectatic OS	–	2/4 (50%)	1/4 (25%)	1/4 (25%)	19% (±9%)

Table 2
Semi-quantitative assessment of NELL-1 immunohistochemistry, by tumor grade.

Tumor grade	*Staining intensity (% of cases stained)*				*Staining distribution*
	0	1+	2+	3+	Mean % of cells stained (±SD)
Benign	–	–	2/10 (20%)	8/10 (80%)	73% (±10%)
Low Grade OS[b]	–	3/4 (75%)	1/4 (25%)	–	21% (±7%)[c]
High Grade OS[a]	–	3/20 (15%)	9/20 (45%)	8/20 (40%)	43% (±26%)[c]

[a] $p < 0.05$ in comparison to benign tumor staining intensity.
[b] $p < 0.01$ in comparison to benign tumor staining intensity.
[c] $p < 0.01$ in comparison to benign tumor staining distribution.

Next, subtypes of OS were examined, including parosteal, periosteal, and telangiectatic OS. Examination of parosteal OS specimens revealed a characteristic dual lineage tumor with alternating zones formed bony trabeculae and fibroblastic stroma (Fig. 3). A relatively paucity in NELL-1 immunostaining was observed among parosteal OS samples. Specifically, weak to intermediate staining intensity in a minority of parosteal tumor cells was seen (1-2+, 28% of tumor cells). Examination of periosteal OS revealed characteristic feather-like osteoid surrounded by zones of intermediate grade chondroblastic differentiation. Surprisingly, NELL-1 immunoreactivity was seen in areas with chondroblastic morphology, but was essentially absent in all areas of ossification. Finally, telangiectatic osteosarcoma specimens were examined which demonstrated a characteristic appearance of highly atypical neoplastic cells in a background of blood, fibrin and sparse osteoid. Among telangiectatic OS specimens, variable intensity of NELL-1 immunoreactivity was noted in a minority of cells (1-3+, 19% of tumor cells). NELL-1 immunostaining was infrequently identified in association with neoplastic osteoid, or more often as rare positivity among single tumor cells.

The intensity and distribution of NELL-1 immunoreactivity is summarized in Tables 1 and 2 Overall, benign bone tumors demonstrated increased intensity of NELL-1 immunostaining ($p < 0.05$) and increased distribution of NELL-1 immunoreactivity ($p < 0.05$). The most variable distribution of NELL-1 staining intensity and distribution was observed among high grade osteosarcoma specimens.

3.4. Correlation of NELL-1 with osteogenic marker expression

In examining the spatial distribution of NELL-1 in benign and malignant bone tumors, a pattern of NELL-1's association with

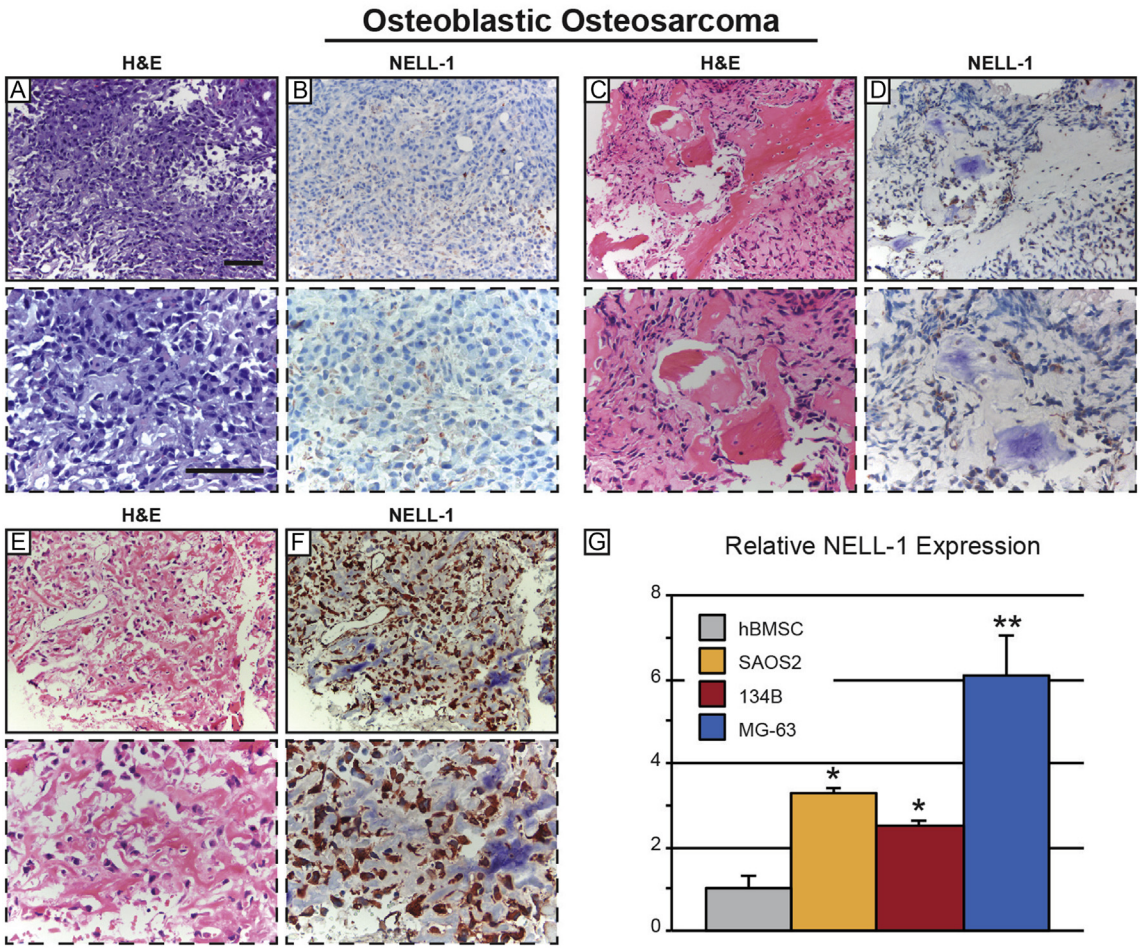

Fig. 2. NELL-1 expression osteoblastic osteosarcoma. (A–F) Appearance of routine H&E staining and NELL-1 immunohistochemical staining in three representative tumors. NELL-1 immunoreactive varies from focal and weak to diffuse and strong. Scale bar: 100 μm. (G) *NELL-1* expression by quantitative RT-PCR among primary human BMSC and osteosarcoma cell lines. $^{*}P < 0.05$, $^{**}P < 0.01$ in comparison to human BMSC.

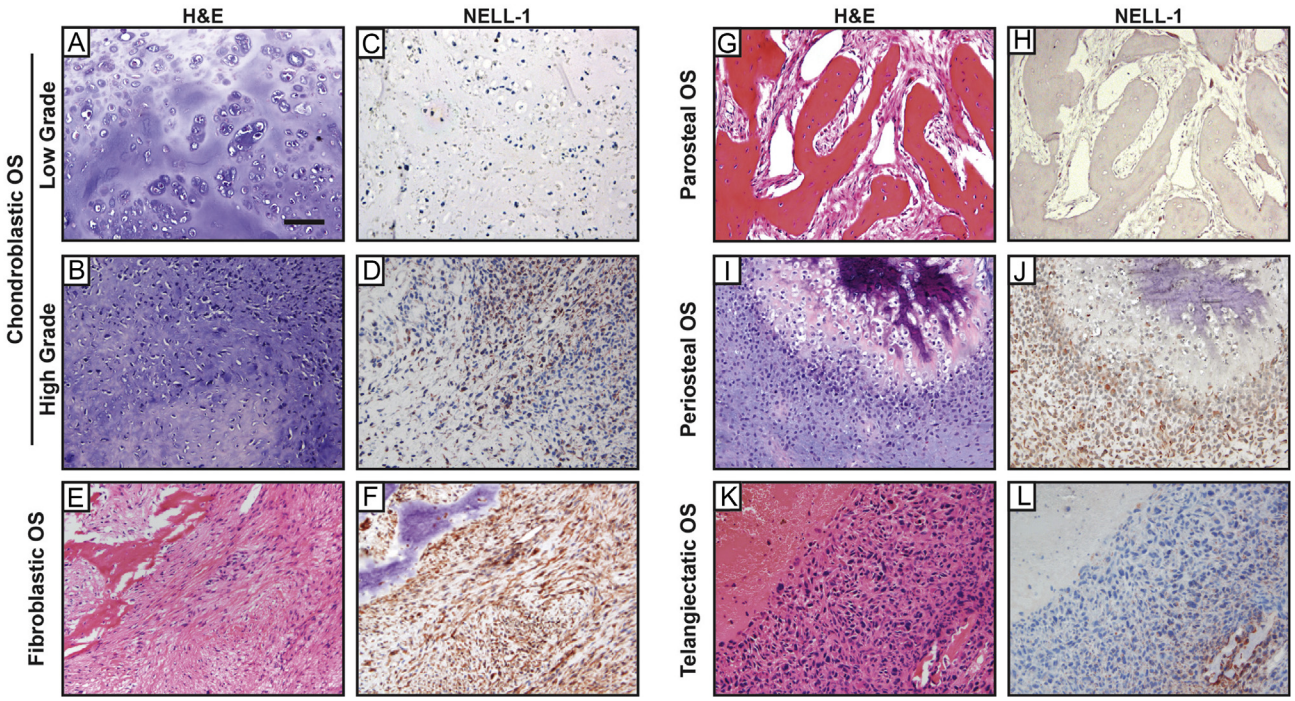

Fig. 3. NELL-1 expression in osteosarcoma subtypes. Appearance of routine H&E staining and NELL-1 immunohistochemical staining in representative osteosarcoma (OS) subtypes. Variants include (A–D) chondroblastic OS, (E,F) fibroblastic OS, (G,H) parosteal OS, (I,J) periosteal OS, and (K,L) telangiectatic OS. Scale bar: 100 μm.

tumor osteoid emerged: NELL-1 seemed to closely associated with osteoid deposition in benign but not malignant bone tumors. To further investigate this, we next sought to correlate NELL-1 expression with traditional markers of osteogenic differentiation, including Bone Sialoprotein (BSP) and Osteocalcin (OCN). Comparisons were based on immunohistochemical staining of serial sections of each tumor (Fig. 4). Benign bone tumors showed clear overlap between NELL-1 immunoreactivity and markers of osteogenic differentiation (*not shown*). In each case, strong and diffuse cellular staining was appreciated. Semi-quantification was performed in which the spatial correlation between NELL-1 and each marker of osteogenesis was assessed using descriptive categories of 'non-concordant,' 'somewhat concordant' and 'concordant' (Tables 3 and 4). All benign bone tumors showed concordance between NELL-1 and BSP immunostaining (10/10, 100%). Similarly, all benign bone tumors showed concordance or 'somewhat concordance' between NELL-1 and OCN immunostaining (4/10 (40%) and 6/10 (60%), respectively). Thus, NELL-1 appeared to be strongly and diffusely expressed in osteoid osteoma and osteoblastoma specimens, and this expression pattern closely approximated the spatial distribution of osteogenic marker expression.

Next, a similar examination was performed across all osteosarcoma specimens, illustrating a strikingly dissimilar pattern (Fig. 4, Tables 3 and 4). In the case of osteoblastic osteosarcoma, the majority of specimens showed only somewhat concordant overlap between NELL-1 and markers of osteogenic differentiation. Tumors with chondroblastic and fibroblastic morphology showed essentially an absence of osteogenic marker expression in areas of NELL-1 immunoreactivity (fibroblastic OS shown). Conversely, both fibroblastic OS and parosteal OS showed areas with BSP + OCN + osteoid, but without NELL-1 immunostaining (parosteal OS shown). Periosteal OS evidenced a marked contrast between distinct zones of NELL-1 expression in chondroblastic areas, in comparison to bone marker expression in ossification centers. Only in the minority of cases was clear-cut overlap between NELL-1 and bone marker immunoreactivity observed among OS samples as a whole (see telangiectatic OS for a rare example of such parallelism in staining). Qualitative analysis of concordance between NELL-1 immunoreactivity and bone marker expression was again performed, by tumor type (Table 3) and tumor grade (Table 4). The vast majority of osteosarcoma specimens showed 'no concordance' or 'somewhat concordance' between NELL-1 and BSP or OCN immunostaining (23/24 tumors for either BSP or OCN). Of note, the single OS specimen which should concordance between NELL-1 and osteogenic markers was noted to be a heavily ossified tumor, with abundant filigree type bone.

4. Discussion

In brief, the present study has identified several unique features of NELL-1 in bone tumor biology. First, NELL-1 expression is increased across all bone-forming skeletal tumors, when compared to non-neoplastic adult bone. Second, NELL-1 is uniformly and markedly increased in the central nidus of the related bone tumors osteoid osteoma and osteoblastoma. In this context, it is unclear if NELL-1 plays a central role in tumor ossification, or whether NELL-1 is a simply a reliable marker for these tumors. Third, NELL-1 has diverse and unpredictable expression patterns in osteosarcomas. However in nearly all cases, NELL-1 immunoreactivity is reduced in OS specimens and does not spatially correlate with osteoid deposition nor traditional markers of osteogenic differentiation.

The expression of NELL-1 in osteosarcomas raises intriguing questions regarding its role in the basic function in osteosarcoma tumor biology. While still a matter of conjecture, the presence of NELL-1 immunoreactivity distinct from osteoid in OS specimens begs the question of its functional importance in OS. Either the protein itself has not induced an osteogenic stimuli, or the neoplastic cell population has not received this cue. More study both isolating tumor-derived NELL-1 protein and supplementing NELL-1 in OS cells in culture is needed to answer these questions.

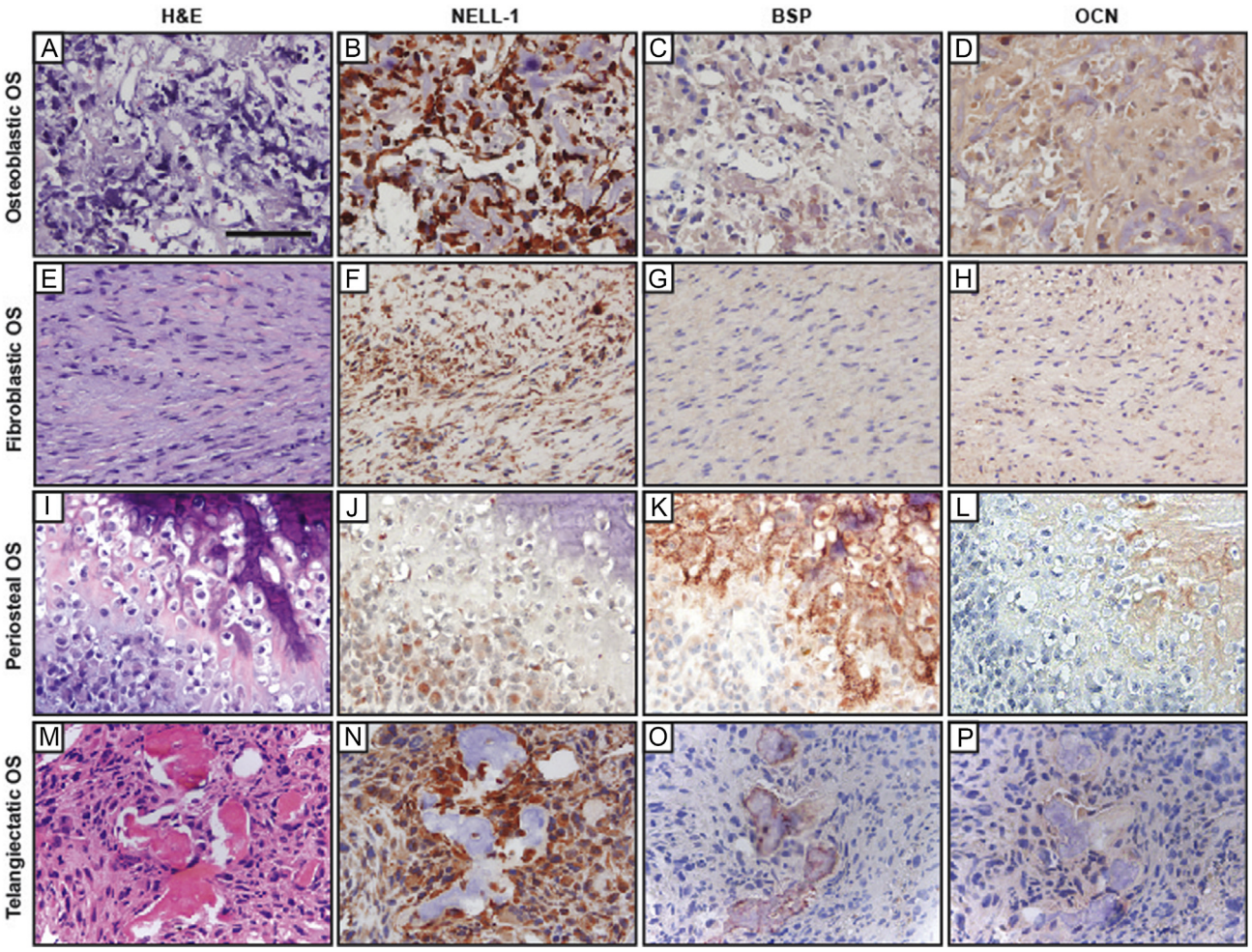

Fig. 4. Correlation of NELL-1 expression with markers of osteogenic differentiation. Appearance of routine H&E staining, NELL-1 immunohistochemical staining, Bone Sialoprotein (BSP) and Osteocalcin (OCN) immunohistochemical staining in serial sections of representative bone tumors. From top to bottom, tumors include: (A–D) Osteoblastic Osteosarcoma (OS), (E–H) Fibroblastic OS, (I–L) Periosteal OS, and (M–P) Telangiectatic OS. Scale bar: 100 μm.

Table 3
Concordance of NELL-1 and osteogenic marker immunohistochemistry, by tumor type.

Tumor type	*Bone Sialoprotein (BSP)*			*Osteocalcin (OCN)*		
	Non-concordant	Somewhat concordant	Concordant	Non-concordant	Somewhat concordant	Concordant
Osteoid Osteoma	–	–	5/5 (100%)	–	3/5 (60%)	2/5 (40%)
Osteoblastoma	–	–	5/5 (100%)	–	3/5 (60%)	2/5 (40%)
Conventional OS	6/11 (55%)	4/11 (36%)	1/11 (9%)	5/11 (45%)	5/11 (45%)	1/11 (9%)
Parosteal OS	3/7 (43%)	4/7 (57%)	–	6/7 (86%)	1/7 (14%)	–
Periosteal OS	1/1 (100%)	–	–	1/1 (100%)	–	–
Telangiectatic OS	1/4 (25%)	3/4 (75%)	–	3/4 (75%)	1/4 (25%)	–

Prior studies in the Saos2 osteosarcoma cell line do suggest that recombinant NELL-1 protein does induce osteogenic differentiation in this cell line via potentiation of MAPK signaling [27]. However, clear variability exists between OS cell lines in their responsiveness to osteoinductive protein stimuli, such as with BMPs (Bone Morphogenetic Proteins) [28]. In addition, NELL-1 does have other known key functions in osteoprogenitor cells. For example, we recently described a key role for NELL-1 induction of osteoblastic cell attachment via binding to the cell surface receptor integrin $\beta1$[4]. This *in vitro* induction of cell attachment may have important

Table 4
Concordance of NELL-1 and osteogenic marker immunohistochemistry, by tumor grade.

Tumor grade	*Bone Sialoprotein (BSP)*			*Osteocalcin (OCN)*		
	Non-concordant	Somewhat concordant	Concordant	Non-concordant	Somewhat concordant	Concordant
Benign	–	–	10/10 (100%)	–	6/10 (60%)	4/10 (40%)
Low grade OS[a,b]	2/4 (50%)	2/4 (50%)	–	4/4 (100%)	–	–
High grade OS[a,b]	10/20 (50%)	9/20 (45%)	1/20 (5%)	12/20 (60%)	7/20 (35%)	1/20 (5%)

[a] $p < 0.01$ in comparison to benign tumor concordance in BSP staining.
[b] $p < 0.01$ in comparison to benign tumor concordance in OCN staining.

correlates to *in vivo* osteosarcoma cell-matrix adhesion, although the extent of this parallelism is as yet unknown.

Several limitations exist for broader extrapolation of the results from the present study. First, we rely on immunohistochemical based detection of NELL-1. Clinical samples vary in their processing, with variable lengths of ischemic time, fixation time, and decalcification time. How these factors influence the NELL-1 antigen is not yet known. Second, the present study is a survey of NELL-1 expression benign and malignant bone tumors, and as such has a modest sample size of any given tumor type. Nevertheless, the present study highlights the presence of NELL-1 across benign and malignant bone tumors. NELL-1 is uniformly and markedly increased in the related bone tumors osteoid osteoma and osteoblastoma and correlates with markers of osteogenic differentiation. In contrast, NELL-1 has diverse and unpredictable expression patterns in osteosarcomas, which suggest a biologic role outside of tumor ossification.

Conflict of interest

Drs. XZ, KT, and CS are inventors of NELL-1 related patents. Drs. XZ, KT, and CS are founders and or board members of Bone Biologics Inc. which sublicenses NELL-1 patents from the UC Regents, which also hold equity in the company.

Acknowledgments

Funding was provided by the UCLA Department of Pathology and Laboratory Medicine and the Translational Research Fund. The authors thank AS James for his excellent technical assistance.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.03.040.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.040.

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