

# A Novel, Nuclear Pore-Associated, Widely Distributed Molecule Overexpressed in Oncogenesis and Development

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**Nuclear pore complexes are large, elaborate macromolecular structures that mediate the bidirectional nucleocytoplasmic traffic. In vertebrates, nuclear pore complexes comprise 50 to 100 proteins termed nucleoporins (Nup). An 88-kd nucleoporin (Nup88) has been recently cloned and characterized, and found to be associated in a dynamic subcomplex with the oncogenic nucleoporin CAN/Nup 214. We have produced a polyclonal antiserum to Nup88, and found that it immunoreacts convincingly in conventional tissue sections of 214 samples of malignant tumors of many types. All carcinomas were stained irrespective of site or line of differentiation; the majority of cases reacted strongly and extensively. *In situ* carcinomas and highly dysplastic epithelia were similarly reactive. Samples of malignant mesotheliomas, gliomas, sarcomas, and lymphoreticular tumors were also stained. Substantial reactions were also found in certain fetal tissues. Focal reactions were noted in some reactive-proliferative processes. Most benign epithelial and mesenchymal tumors and hyperplasias, and normal adult tissues reacted weakly and sporadically or not at all. Immunoblot analysis of selected samples strongly corroborated those findings. If further substantiated, our findings indicate that Nup88 could be regarded as a selective yet broadly based proliferation marker of potential significance in the histological evaluation and diagnosis of malignant transformation. Its ready applicability on conventional paraffin sections and on cytological preparations may broaden its clinical and investigative significance. (Am J Pathol 2000, 157:1605–1613)**

In 1993, a monoclonal antibody directed against *Candida krusei* cytochrome *c* was shown to react with a cytoplasmic fraction protein of a human lung carcinoma cell line.<sup>1</sup> Independently, it was reported that monoclonal antibody C6, generated against *Candida albicans* mannoproteins,

reacted specifically with a M<sub>r</sub> 43,000 molecule from samples of human ovarian carcinoma but not with nonneoplastic counterpart cells.<sup>2</sup> Subsequently, we showed that this monoclonal antibody recognized in immunoblots an additional protein of M<sub>r</sub> 88,000.<sup>3</sup> Using a cDNA library, we recognized the mammalian molecule as nucleoporin (Nup) 88<sup>3</sup>; and, identified it as a protein located at the nuclear membrane known to be involved in nuclear-cytoplasmic transport.<sup>4,5</sup> In turn, Nup88 had been found to be associated with the central domain of CAN/Nup214, a nuclear pore complex component putatively implicated in nuclear protein import, nuclear mRNA export, and the regulation of the cell cycle.<sup>6</sup> Notably, the CAN/Nup214 proto-oncogene is involved in chromosomal rearrangements related to two variants of leukemia.<sup>7,8</sup>

As part of the investigation that led to the recognition that the molecule bound by monoclonal antibody C6 corresponded to Nup88, a polyclonal antiserum directed to the pertinent recombinant protein was generated. By immunohistochemistry, this antiserum was shown to recognize several human tumor cell lines as well as ovarian carcinomas in tissue sections; parallel results were obtained by immunoblot analysis.<sup>3</sup> We now report that this antiserum immunostains richly and extensively conventional histological sections of 214 samples representing a wide spectrum of malignant tumors including carcinomas and sarcomas of diverse sites, lineages, and differentiation as well as some mesotheliomas, gliomas, melanomas, and lymphoreticular neoplasms. Certain fetal tissues were similarly stained. Notably, benign neoplasms and reparative processes showed only focal and less intense reactions whereas normal adult cells reacted only sporadically and weakly. Immunoblots of selected samples showed parallel results. Our findings indicate that this molecule most probably corresponds to Nup88. Its significant overexpression and its exceedingly wide distribution across a wide spectrum of cancers and precancerous dysplasias raise the possibility of using it as a broadly based generic histodiagnostic marker of malignant transformation.

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## Materials and Methods

### Samples

Cases were selected on the basis of known diagnoses from the files of the Rush-Presbyterian-St. Luke's Medical Center, Chicago, and the Hospital of the Faculty de Medicina, Bilbao, Spain. A total of 214 malignant tumors were examined; benign tumors, hyperplasias, and related conditions were also included (see Table 1). Most of these cases had been extensively studied and characterized in previous studies.<sup>9-13</sup> Surgical procedures were performed with due consent, and were based on widely accepted therapeutic and/or diagnostic protocols. Autopsy samples from adult and fetal cases were obtained from Rush-Presbyterian-St. Luke's Medical Center; autopsies were performed based on duly obtained consent. The anonymity of the patients was protected in all cases.

### Immunohistochemistry

Two paraffin blocks per case were selected; diagnoses were confirmed on conventional hematoxylin and eosin-stained sections by two independent observers (VEG and AO). All tissues had been fixed in formalin, conventionally processed, and embedded in paraffin. Sections for immunostaining were cut at 4  $\mu\text{m}$ , set on coated slides, and placed on a warmer at 60°C for 1 hour; subsequently, they were deparaffinized in xylene and rehydrated in graded alcohols. No pretreatment with microwave or enzymes before exposure to the primary antiserum was applied. Details on the preparation and characteristics of the antiserum have been recently published.<sup>3</sup> Staining was accomplished by the avidin-biotin-peroxidase method as originally outlined by Hsu et al<sup>14</sup>; commercial reagents were used (DAKO, Carpinteria, CA). Best results were obtained when the antiserum was applied overnight in a humid chamber at 4°C at a concentration of 1/500; the diluent was that provided in a commercial kit (Ventana Medical Systems, Tucson, AZ). Binding sites were visualized with 3,3'-diaminobenzidine (Aldrich Chemicals, Danvers, MA); in the case of melanocytic lesions, alkaline phosphatase (DAKO) was used as chromogen. All sections were counterstained with hematoxylin to improve nuclear visualization. As negative controls, slides were similarly processed but omitting the primary antiserum. Staining intensity was rated as weak, moderate, or strong; in heterogeneous cases the rating of the predominant pattern prevailed. The extent of the reaction was defined by the percentage of reactive cells and graded as negative (0) to 1+ to 5+ as previously described.<sup>15</sup>

### Immunoblots

For immunoblot analysis, freshly obtained samples were placed in vials containing precooled isopentane, and snap-frozen in liquid nitrogen. All samples were kept in a deep freezer at -80°C until used. Tumors were homogenized in 1% sodium dodecyl sulfate-0.14 mol/L  $\beta$ -mer-

captoethanol and then centrifuged over a Qiashraded (Qiagen, Germany) column to shear the DNA. The protein content of the samples was calculated with the DC protein assay system (Bio-Rad, Richmond, CA) and 15  $\mu\text{g}$  of proteins were separated on 10% polyacrylamide gels. Proteins were electrotransferred to polyvinylidene difluoride membranes, blocked with nonfat milk in phosphate-buffered saline, and incubated with our polyclonal antiserum at a dilution of 1:500. After washing, the membranes were reacted with a horseradish peroxidase-labeled goat anti-rabbit, washed again, and the reacting bands were revealed with the Amersham enhanced chemoluminescence system (Amersham, Buckinghamshire, UK).

## Results

### Immunohistochemistry

All malignant neoplasms stained convincingly; most reactions were moderate to strong and 3+ to 5+ in extent. *In situ* carcinomas were invariably stained as were, albeit less so, dysplastic lesions that did not reach the level of carcinoma *in situ*. Benign tumors showed variably extensive and generally weak immunoreactivity. Salient points are summarized in Table 1.

Gastric carcinomas were extensively and strongly stained. The contrast between the surrounding or overlying mostly negative mucosa and the convincingly reactive tumor was evident (Figures 1, a and b). Reactions were similar in tumors showing variably differentiated glands, solid clusters, or linitis plastica single-cell pattern. One case showed intestinal metaplasia with some dilated glands as well as *in situ* carcinoma with the latter reacting strongly (Figure 1c). The immunostaining was characteristically granular; the granules were rather large as compared with the delicate dots seen in synaptophysin reactions.<sup>16,17</sup> In a minority of cells, a perinuclear localization was noted but the distribution was predominantly cytoplasmic (see below). The cell membrane did not stain.

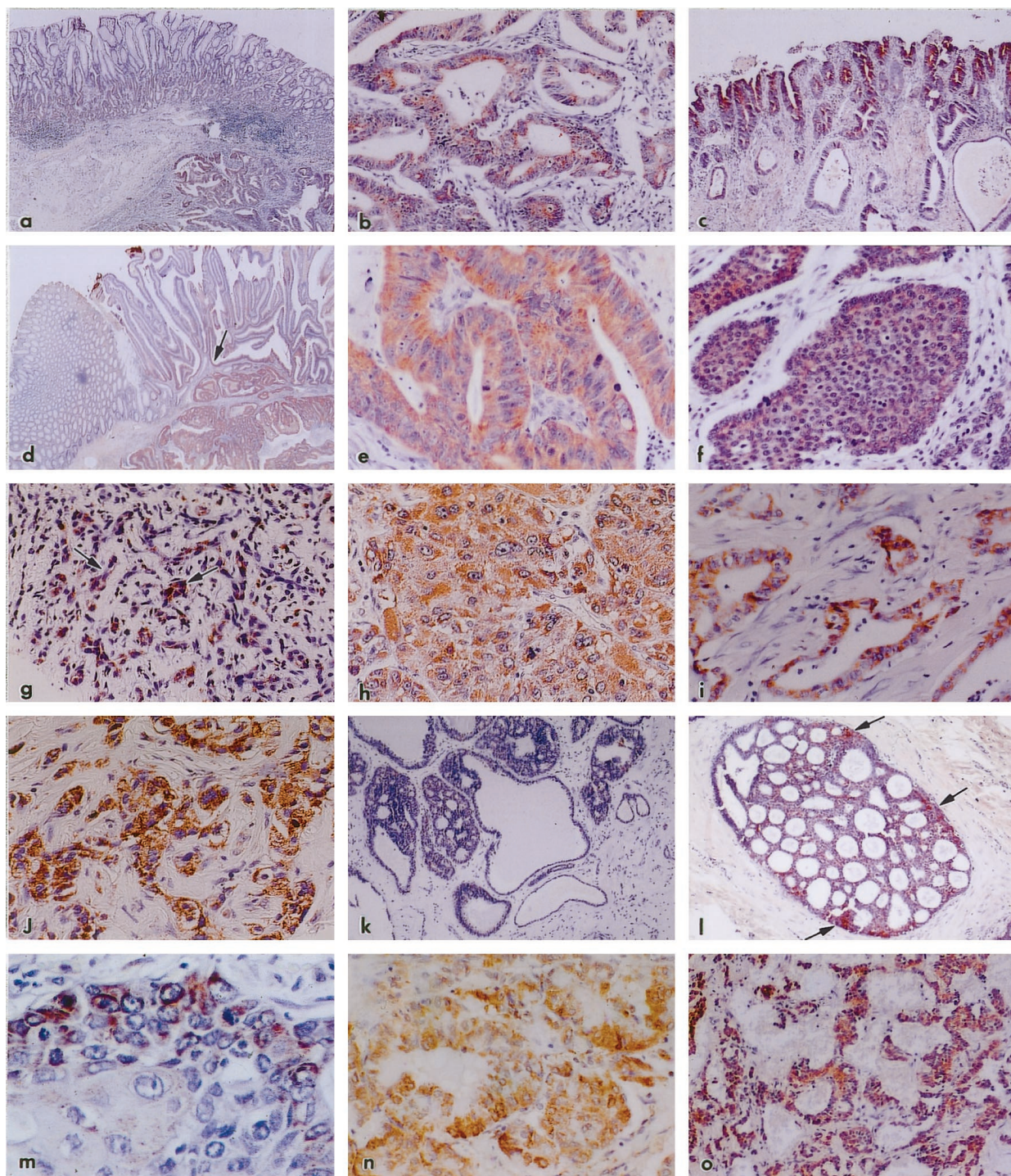
Colonic adenocarcinomas were strongly and diffusely stained. Several blocks included normal mucosa, polyps with variable degrees of dysplasia, and infiltrating carcinoma. In these instances, the normal mucosa was negative, the polyps reacted paralleling the degree and extent of dysplasia, and the overt carcinomas reacted strongly (Figure 1, d and e). Two carcinomas of mixed exocrine and neuroendocrine phenotypes and two pure neuroendocrine carcinomas were similarly stained (Figure 1f). In polyps not associated with carcinoma, villous adenomas stained stronger and more extensively than their tubular counterparts; foci of intramucosal carcinoma stained convincingly. Hepatocellular carcinomas were strongly reactive. One of these developed in the background of advanced cirrhosis; in this case, occasional cells in proliferating bile ductules in cirrhotic septa were weakly stained (Figure 1g) whereas the adjacent carcinoma stained diffusely and strongly (Figure 1h), and dysplastic nodules reacted moderately. Pancreatic car-

**Table 1.** Nup88 Immunoreactivity of Tumors and Related Conditions

Site and diagnosis	No. of cases and reaction	Extent of reaction*	Intensity of reaction†
Epithelial tumors			
Stomach			
Infiltrating adenocarcinoma	11/11	3+/5+	m/s
<i>In situ</i> carcinoma	2/2	3+/4+	m/s
Colon			
Infiltrating adenocarcinoma	12/12	3+/5+	m/s
<i>In situ</i> adenocarcinoma	3/3	2+/4+	m
Villous adenoma	3/3	2+/3+	m
Tubular adenoma	5/5	2+/3+	w/m
Neuroendocrine carcinoma	2/2	3+/4+	m/s
Liver			
Hepatocellular carcinoma	4/2	3+/5+	m/s
Dysplastic nodules	2/2	2+/3+	w
Pancreas			
Adenocarcinoma	7/7	3+/4+	m/s
Neuroendocrine carcinoma	3/3	3+/4+	m/s
Breast			
Infiltrating ductal carcinoma	14/14	3+/5	m/s
Infiltrating lobular carcinoma	12/12	3+/5+	m/s
<i>In situ</i> ductal carcinoma	16/16	3+/4+	m
<i>In situ</i> lobular carcinoma	5/5	3+	m
Fibroadenoma	2/5	1+	w
Fibrocystic disease	16/28	1+/3+	w/m
Lung			
Squamous carcinoma	8/8	3+/5+	m/s
Adenocarcinoma	12/12	3+/5+	m/s
Bronchioloalveolar carcinoma	2/2	3+/4+	m
Large-cell carcinoma	3/3	3+/5+	m/s
Neuroendocrine carcinoma	14/14	3+/5+	m/s
Carcinoid	9/9	2+/4+	m
Hyperplastic bronchi	3/3	2+/3+	w/m
Ovary			
Cystadenoma	2/3	2+	w/m
Benign teratoma	1/1	2+	w/m
Borderline serous carcinoma	2/2	2+/3+	m
Borderline mucinous carcinoma	2/2	2+/3+	m
Serous carcinoma	6/6	3+/5+	m/s
Mucinous carcinoma	4/4	3+/5+	m/s
Endometrioid carcinoma	1/1	4+	m/s
Clear-cell carcinoma	2/2	3+/5	m/s
Uterus			
Endometrial carcinoma	12/12	3+/5+	m/s
Endometrial hyperplasia	4/10	0/2+	w
Prostate			
Adenocarcinoma	11/11	3+/5+	m/s
PIN, high grade	6/6	2+/3+	m/s
PIN, low grade	2/4	1+/2+	w/m
Benign glandular hyperplasia	2/6	(+)/2+	w/m
Kidney			
Clear cell carcinoma	4/4	3+/5+	m/s
Adrenal			
Cortical adenoma	2/2	2+/3+	w
Mesenchymal tumors			
Fibrosarcoma	5/5	3+/4+	m
Malignant fibrous histiocytoma	7/7	3+/4+	w/m
Kaposi sarcoma	2/2	3+/4+	m
Dermatofibrosarcoma protuberans	5/5	3+/4+	m
Giant-cell tumor, benign	0/2	0	—
Leiomyoma	2/2	3+/4+	w
Atypical fibroxanthoma	2/2	2+/3+	w
Angiolipoma	1/2	(+)	w
Miscellaneous tumors			
Large-cell lymphoma	3/3	3+/4+	m
Lymphoblastic lymphoma	1/1	3+/4+	m
Hodgkin's disease	4/4	2+/3+	m
Malignant mesothelioma	5/5	3+/5+	m/s
Benign mesothelioma	0/2	0	—
Glioblastoma multiforme			
Malignant melanoma	4/4	3+/5+	m/s
Infiltrating	4/4	3+/5+	m/s
<i>In situ</i>	2/2	3+/4+	m/s

\* (+) = <1% positive cells; 1+ = 1–5% positive cells; 2+ = 6 to 15% positive cells; 3+ = 16 to 50% positive cells; 4+ = 51 to 95% positive cells; 5+ = 95% positive cells.

† S, strong; m, moderate; w, weak.



**Figure 1.** All samples were immunostained with polyclonal antiserum to Nup88; the chromogen is DAB. **a:** Stomach adenocarcinoma; the prominent mucosa (**top**) is not reactive, whereas the carcinoma invading the submucosa (**bottom right**) is clearly stained. Original magnification,  $\times 60$ . **b:** Stomach adenocarcinoma; higher magnification of tumor in **a** shows diffuse and strong staining of the malignant glands. Original magnification,  $\times 480$ . **c:** Stomach with extensive intestinal metaplasia; a strongly and diffusely reactive *in situ* carcinoma is evident. Original magnification,  $\times 180$ . **d:** Colon adenocarcinoma; the normal mucosa on the **left** is negative whereas the basal aspect of the glands of a villous adenoma are moderately reactive (**arrow**). The overt carcinoma invading the submucosa is richly stained. Original magnification,  $\times 120$ . **e:** Colon adenocarcinoma; higher magnification of tumor in **d** shows diffuse, strong, and granular reaction of malignant glands. Original magnification,  $\times 620$ . **f:** Colon; neuroendocrine carcinoma showing portions of characteristically organoid clusters diffusely reactive for Nup88. Original magnification,  $\times 480$ . **g:** Liver; cirrhotic septum in the vicinity of the carcinoma depicted in **h**. Note focal staining in proliferating bile ductules (**arrows**) amid nonreactive fibroconnective tissue. Original magnification,  $\times 180$ . **h:** Liver; hepatocellular carcinoma; higher magnification shows strong, extensive, and granular reaction. Original magnification,  $\times 620$ . **i:** Pancreas; adenocarcinoma showing strong Nup88 staining of malignant glands. Original magnification,  $\times 420$ . **j:** Breast; infiltrating ductal carcinoma showing strongly Nup88 reactive clusters. Original magnification,  $\times 420$ . **k:** Breast; fibrocystic changes; hyperplastic and cystic ducts are nonreactive. Original magnification,  $\times 120$ . **l:** Breast; intraductal carcinoma, cribriform variant. The staining is diffuse but is best appreciated toward the periphery of the duct (**arrows**). Original magnification,  $\times 120$ . **m:** Lung; well-differentiated squamous carcinoma; part of neoplastic cluster showing strong staining particularly of the peripheral cells; the central, more mature cells approaching pearl formation show fewer reactive granules. Original magnification,  $\times 620$ . **n:** Lung; moderately differentiated adenocarcinoma; note strong and diffuse reaction. Original magnification,  $\times 480$ . **o:** Bronchial carcinoid. Note moderately but diffusely reactive cell ribbons and clusters amid negative stroma. Original magnification,  $\times 180$ .

cinomas of variable glandular differentiation showed consistent, moderate to strong reactions (Figure 1i); two neuroendocrine carcinomas were similarly stained. Regardless of tumor phenotype, nonneoplastic exocrine and neuroendocrine cells in the vicinity of, or entrapped within, tumors were occasionally, focally reactive.

All infiltrating breast carcinomas of ductal (Figure 1j) or lobular types reacted convincingly, whereas ducts nearby, or entrapped within them, reacted weakly and sporadically or not at all. Mucinous (colloid) carcinomas showed strong staining of the scanty malignant cells whereas the dominant mucous pools did not react. In several carcinomas, the strongest staining was noted in the peripheral, invasive edge of the tumors. Fibrocystic changes in the vicinity of carcinomas including cysts, ductal hyperplasia, adenosis, papillomas, and apocrine metaplasia, showed weak to moderate, focal reactions, whereas similar changes not associated with carcinomas ranged from focally reactive to entirely negative (Figure 1k). Atypical ductal hyperplasia and all variants of ductal carcinoma *in situ* stained convincingly (Figure 1l), fibroadenomas reacted weakly and focally or not at all.

Pulmonary squamous, adeno-, bronchioloalveolar, large-cell, and neuroendocrine carcinomas reacted richly and extensively (Figure 1, m and n); in well-differentiated squamous carcinomas, the peripheral growing edge of the clusters reacted strongly whereas the central cells approaching the squamous pearls were less reactive. Hyperplastic bronchi in the vicinity or within tumors were often stained. Bronchial carcinoids stained moderately and often extensively (Figure 1o). Ovarian carcinomas including serous, mucinous, clear-cell, and endometrioid types were convincingly stained; borderline tumors stained moderately whereas their invasive (Figure 2a) and particularly their high-grade counterparts were strongly reactive. Benign cystadenomas showed uneven, generally weak reactions. In a benign teratoma, the basal cells of the skin and adnexa, the respiratory epithelium and cartilaginous cells showed moderate staining. Endometrial carcinomas of all types reacted strongly (Figure 2b). Several of these cases were associated with endometrial hyperplasia; and, although the carcinomas stained convincingly, the bland variants of hyperplasia did not (Figure 2c). Also interesting was that the luminal aspect of several tumors stained less intensely than the deep, invasive aspect. In several cases, segments of fallopian tubes included in these samples showed focal, moderate reactions, as did cells comprising small follicular cysts and corpora lutea.

All prostatic carcinomas reacted strongly and diffusely irrespective of degree of differentiation (Figure 2g) whereas nonneoplastic ducts and acini entrapped within them stained weakly and sporadically or not at all. In the case of poorly differentiated and hypernephroid carcinomas, minute clusters or single reactive cells were readily detected in the stroma and, around and/or within nerves (Figure 2e). Foci of low- and high-grade prostatic intraepithelial neoplasia were consistently stained, particularly the latter. Benign hyperplastic and atrophic glands were either negative (Figure 2f) or showed rare positive cells. All renal carcinomas examined were of clear cell

type and all reacted strongly (Figure 2g); some reactive tubules in the vicinity stained as well but weakly. Two adrenocortical adenomas showed weak but fairly extensive reactions.

### *Mesenchymal Tumors*

Fibrosarcomas, malignant fibrous histiocytomas (Figure 2h), and Kaposi sarcomas stained moderately to strongly, and a single leiomyosarcoma reacted strongly in both primary and metastatic sites (Figure 2i). Dermatofibrosarcoma protuberans, benign giant cell tumors, atypical fibroxanthomas, and angioliipomas showed focal, moderate to weak reactions. Two (uterine) leiomyomas stained rather extensively but weakly.

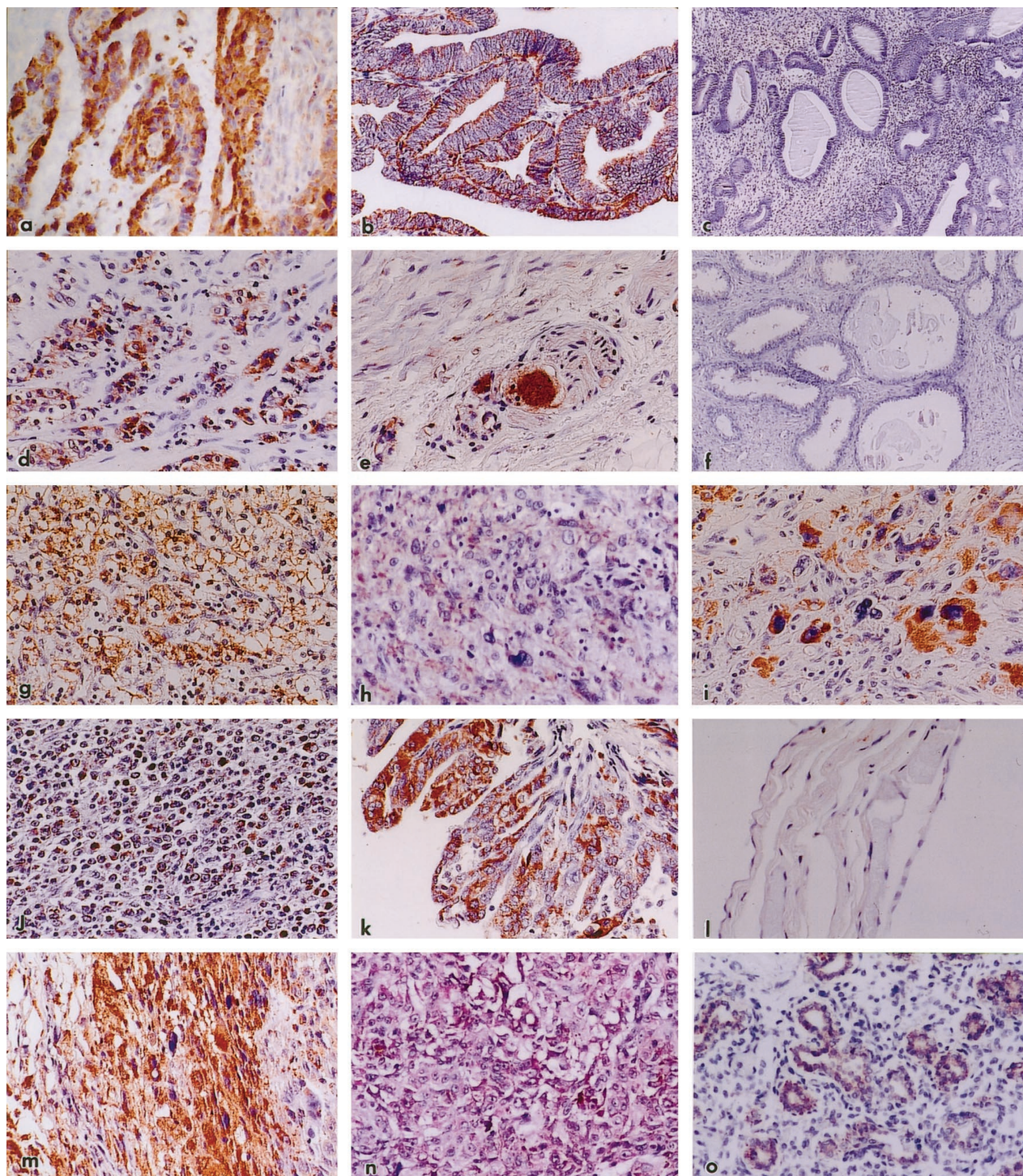
### *Miscellaneous Tumors and Fetal Tissues*

Diffuse large cell lymphomas and a lymphoblastic lymphoma stained strongly and extensively (Figure 2j). In several cases of Hodgkin's disease, the Reed-Sternberg as well as the lacunar cells stained convincingly whereas the associated, nonneoplastic leukocytes did not. Malignant mesotheliomas stained strongly (Figure 2k); similar reactions were seen in epithelial, sarcomatoid, and biphasic variants. In contrast, samples from two benign cystic mesotheliomas (multiple peritoneal inclusion cysts) were consistently negative (Figure 2l). Several small samples of glioblastoma multiforme stained strongly and extensively (Figure 2m). *In situ* malignant melanomas showed convincing reactions of the dysplastic melanocytes at the base of the epidermis, and of single cells migrating upwards in the epidermis. Invasive malignant melanomas of various sites showed intense and diffuse reactions (Figure 2n) irrespective of architecture or melanin content.

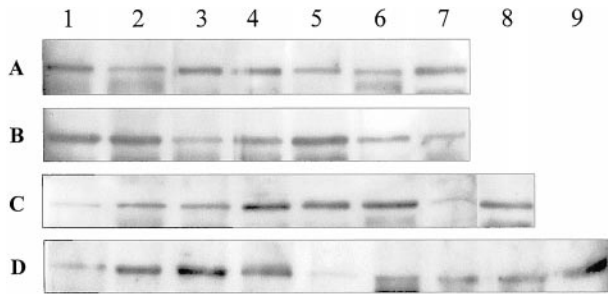
Fetal samples examined ranged from 20 to 24 weeks of gestational age; tissues included lung, heart, liver, kidney, and bowel. Reactions were particularly convincing in developing bronchi and primitive air spaces (Figure 2o) as well as in the crypts of the intestinal mucosa.

### *Immunoblots*

The results of these experiments are shown in Figure 3. With a single exception, all samples of malignant tumors analyzed, showed a convincing increase in the amount of Nup88. All colon carcinomas (A1–A5) showed reactive bands that were far stronger than the controls; the comparatively weak band noted in A2 may reflect this tumor's extensive necrosis. In the case of lung tumors, the respectable band of the bronchial carcinoid (B1) corresponds in fact to a rather intensely reactive tumor (Figure 1o). Notice also the intense B2 and B4 bands (carcinomas) and compare with the meager B3 band that represents a carcinoma treated with radio- and chemotherapy before removal resulting in extensive tumor necrosis. The strong B5 band pertains to a leiomyosarcoma metastatic to the lung (Figure 2i), and the relatively weak but distinct B6 band corresponds to bronchi in the vicinity of squa-



**Figure 2.** The same antiserum was used in all samples. The chromogen was DAB except for **n** for which alkaline phosphatase (red) was used. **a:** Ovary; moderately differentiated papillary serous carcinoma; note rich, diffuse reaction of neoplastic papillae. Original magnification,  $\times 480$ . **b:** Endometrium; adenocarcinoma, well differentiated, showing strong and diffuse Nup88 reactivity. Original magnification,  $\times 480$ . **c:** Endometrium; cystic hyperplasia; no reaction is evident. Original magnification,  $\times 180$ . **d:** Prostate; adenocarcinoma, Gleason 4 + 5; intense reaction involving the majority of malignant cells is seen. Original magnification,  $\times 420$ . **e:** Prostate; periphery of carcinoma shown in **d**. Small clusters of malignant cells are strongly stained; note also the intense reaction of neoplastic cells around and within a nerve. Original magnification,  $\times 540$ . **f:** Prostate showing dilated, hyperplastic, and atrophic glands; no Nup88 reaction is seen. Original magnification,  $\times 180$ . **g:** Kidney; clear cell carcinoma. A strong and extensive reaction is evident. Original magnification,  $\times 480$ . **h:** Soft tissue; malignant fibrous histiocytoma; a weak to moderate but extensive reaction involves most neoplastic cells. Original magnification,  $\times 480$ . **i:** Leiomyosarcoma metastatic to the lung (see Figure 3, immunoblot B5). Strong reactivity is noted particularly in the bizarre giant cells. Original magnification,  $\times 620$ . **j:** Lymph node; large cell lymphoma diffusely reactive for Nup88. Original magnification,  $\times 160$ . **k:** Pleura; malignant papillary mesothelioma richly and diffusely reactive for Nup88. Original magnification,  $\times 480$ . **l:** Abdomen; benign cystic mesothelioma; no reaction is seen. Original magnification,  $\times 480$ . **m:** Brain; glioblastoma multiforme; a rich and extensive reaction is noted. Original magnification,  $\times 480$ . **n:** Skin; malignant melanoma immunostained for Nup88 with alkaline phosphatase as chromogen (red). A rich and extensive reaction is evident. Original magnification,  $\times 480$ . **o:** Fetal lung (20 weeks, 360 g). A moderate immunoreaction involves the primitive air spaces whereas the stroma appears nonreactive. Original magnification,  $\times 320$ .



**Figure 3.** Immunoblot analysis of Nup88 content in neoplastic, hyperplastic, and normal tissues. Total protein extracts of colon (A), lung (B), breast (C), and ovary (D) were prepared as described in Materials and Methods with the polyclonal antiserum to Nup 88. **A:** Colon. **Lanes 1, 3, 4, and 5** represent adenocarcinomas; **lane 2** represents also an adenocarcinoma but showing extensive necrosis; **lane 6** is the negative control consisting of normal human lymphocytes whereas **lane 7** is the positive control representing an ovarian carcinoma known to express abundant Nup88 from previous studies. **B:** Lung. **Lane 1** represents the bronchial carcinoid depicted in Figure 1o. **Lanes 2 and 4** represent an adenocarcinoma and a squamous carcinoma, respectively, whereas **lane 3** is a poorly differentiated squamous carcinoma treated with chemotherapy and radiotherapy before excision. The strongly reactive **lane 5** represents a metastatic leiomyosarcoma shown in Figure 2i; **lane 6** represents hyperplastic bronchi in the vicinity of but not involved by a carcinoma. Negative control in **lane 7** as in A. **C:** Breast. **Lane 1** represents typical, benign fibrocystic disease depicted in Figure 1k. **Lanes 2 and 3** represent fibroadenomas; **lanes 4 and 5** represent infiltrating ductal carcinomas whereas **lane 6** is an infiltrating lobular carcinoma. **Lane 7** is a normal control adult female breast and **lane 8** is a known, positive control ovarian carcinoma. **D:** Ovary. **Lane 1** represents a benign mucinous cystadenoma; **lanes 2 and 3** represent papillary serous carcinomas, and **lane 4** is a metastasis from a gastric signet-ring cell carcinoma. **Lane 5** shows a surprisingly low signal given by an endometrioid carcinoma possibly reflecting sampling discrepancies. **Lanes 6, 7, and 8** are negative controls and **lane 9** is the positive counterpart.

mous carcinoma showing hyperplastic and metaplastic changes.

The contrast between benign and malignant proliferations in the same organ is well depicted in the series of breast lesions; all carcinomas (C4–C6) show prominent bands whereas fibroadenomas (C2 and C3) and one bland variant of fibrocystic disease (C1, corresponds to Figure 1k) show delicate bands, and the normal breast is virtually negative (C7). In the ovarian samples, the meager band noted in D1 (benign cystadenoma) is in stark contrast with the broad bands seen in the carcinomas (D2–D4). Except for problems related to limited sampling, we cannot explain the rather weak band of the endometrioid carcinoma (D5) that by immunohistochemistry was moderately stained.

Densitometric quantification of the blots showed an increased expression in carcinomas between 1.5 and 5 times greater than the normal controls. Still, it should be considered that these figures might well represent a significant underestimation for they do not take into account the considerable amount of stroma that many of these tumors had. More detailed quantitative analyses should be performed based on larger series, and subsequent to appropriate microdissection.

## Discussion

Our immunohistochemical results strongly reinforced by parallel immunoblot data point to Nup88 as a molecule of exceedingly wide distribution that is consistently overex-

pressed in a broad spectrum of carcinomas as well as malignant mesotheliomas, and a number of sarcomas, melanomas, gliomas, and lymphoreticular tumors. Nup88 was similarly enhanced in severe dysplasias and *in situ* carcinomas of organs such as colon, stomach, breast, and prostate. We also showed distinct Nup88 enhancement during fetal development in sites including pulmonary primitive air spaces and intestinal crypts. Focal expression was also noted in some proliferative-reactive tissues in the vicinity of tumors, eg, hyperplastic bronchial mucosa. Conversely, Nup88 was either sporadic or not detectable in most benign tumors and hyperplasias. In normal adult tissues, Nup88 was occasionally noted in sites such as colonic crypts, bronchial mucosa, and fallopian tubes.

With regard to epithelial cancers, Nup88 enhancement was seen not only across a broad spectrum of sites but through all major differentiation lines. Thus, lung carcinomas with squamous, glandular, and neuroendocrine features were strongly positive. In the gastrointestinal tract and pancreas adeno- and neuroendocrine carcinomas were similarly reactive, whereas in the ovary, serous, mucinous, endometrioid, and clear-cell carcinomas reacted as well. Moreover, Nup88 overexpression seems to be independent of presumed histogenesis as it was found in carcinomas from diverse organs derived from all embryonal layers. In addition, as noted earlier,<sup>3</sup> we also found that in sites such as the ovary, high-grade carcinomas seemed to react more vigorously than their low-grade and borderline counterparts. Furthermore, in some breast and endometrial carcinomas, we noted that the invasive periphery of the tumors stained more strongly than the center. These findings suggest a spatial and temporal relationship between Nup88 overexpression and tumor expansion, and parallel previous observations wherein certain extracellular matrix proteins, eg, tenascin and cellular fibronectins are strongly enhanced in the periphery of carcinomas reflecting areas of active remodeling.<sup>10,18</sup>

Nup88 enhancement was clearly evident in severe epithelial dysplasias and *in situ* carcinomas of the colon, stomach, breast, and prostate; this overexpression was noted irrespective of the presence or absence of an overt synchronous cancer. In contrast, proliferative but benign variants of conditions such as fibrocystic disease of the breast, endometrial hyperplasia, tubular adenomas of the colon, and glandular prostatic hyperplasia showed no significant Nup88 enhancement. Similar contrasts were noted in mesenchymal and other miscellaneous malignancies, eg, strong and extensive reactions in a leiomyosarcoma and in malignant mesotheliomas contrasting with weak and sporadic or absent staining in their benign counterparts. Notably, certain active reparative processes, eg, bile duct proliferation in cirrhosis, and proliferating renal tubules in the vicinity of carcinoma associated with pyelonephritis showed modest but convincing Nup88 reactions. Again, such areas are known to undergo active remodeling as reflected by the enhancement of pertinent matrix molecules.<sup>10,18–21</sup> These findings indicate that enhanced Nup88 reflects a selective cellular

proliferation that is most often, but not exclusively, associated with the malignant or premalignant phenotype.

Some of the above findings of Nup88 overexpression in malignancy coupled with its detection in some fetal tissues suggest parallels between it and a number of oncodevelopmental marker molecules including carcinoembryonic antigen and some related substances. However, the latter molecules are for the most part cell membrane-associated glycoproteins known to have or suspected of having cell-cell adhesive functions.<sup>22,23</sup> In addition, they are selectively expressed in some epithelia but not in others, and are rare in nonepithelial tissues. Moreover, the punctiform, perinuclear, and cytoplasmic localization of Nup88 differs substantially from that of the above molecules. These observations added to those that Nup88 is also significantly enhanced in malignancies as diverse as carcinomas, some sarcomas and lymphomas, mesotheliomas, melanomas, and gliomas point to radical differences between Nup88 and oncodevelopmental markers currently used.

A consistent finding in the present study was the predominantly cytoplasmic localization of the overexpressed protein in the involved cells. Our previous studies on several cell lines showed that most of the protein was located at the nuclear membrane with comparatively small amounts in the cytoplasm. Noteworthy in many neoplastic and some nonneoplastic cells is the presence of aggregates of annulate lamellae<sup>24</sup>; these structures are thought to derive from nuclear membranes,<sup>25</sup> and show features of the latter, eg, components of nuclear pore complexes including nucleoporins as described in *Xenopus* oocytes,<sup>26</sup> and in rat cells wherein they were visualized as cytoplasmic dots.<sup>27</sup> Therefore, we infer that the conspicuous cytoplasmic granules we found may reflect increased complements of annulate lamellae. In this context, it merits mentioning that other oncoproteins may also be aberrantly located, eg, the known nuclear-cytoplasmic mislocation of the BRCA1 gene product in breast carcinomas.<sup>28</sup>

The polyclonal antiserum used in these experiments allowed us to recognize clearly and consistently a  $M_r$  88,000 band in immunoblots; in addition, other weakly reactive bands were at times detected. The precise nature of these additional reactive bands remains unclear, but it should be stated that our antiserum reacts strongly with the glutathione *S*-transferase-Nup88 fusion proteins used for the immunization of the rabbits.<sup>3</sup> We cannot as yet state whether the aforementioned bands of lower molecular weight reflect degradation products of a single molecule or distinct proteins that share a similar or common epitope. The eventual identification of the epitope recognized by our antiserum should help elucidate the fragments of the proteins recognized by it. Given our current data, some questions may be said to persist as to whether the material recognized by our antiserum corresponds in fact to Nup88. Significantly, in carcinomas of various sites and in one sarcoma, strong and extensive immunostaining of tissue sections was paralleled by similarly strong  $M_r$  88,000 reactive bands in Western blots of the same samples; conversely, in samples of hyperplasias, benign tumors, and normal tissues, weak or unde-

tectable immunoreactions were reflected by weakly reactive bands in the corresponding immunoblots. Moreover, no differences in intensity were noted in the additional reactive bands found in tumors as well as controls thus reinforcing the notion that our antiserum is specific for Nup88. These data strongly suggest that the molecule in question corresponds indeed to Nup88.

In vertebrate cells, the nuclear pore complex is a large macromolecular aggregate<sup>29,30</sup> with an estimated molecular mass of  $125 M_r$ ,<sup>31</sup> it includes 50 to 100 proteins termed nucleoporins.<sup>32</sup> On the other hand, in yeast, nuclear pore complexes are smaller, have a molecular mass in the range of  $66 M_r$ , and may include 30 to 40 nucleoporins.<sup>33,34</sup> Among the known nucleoporins, the rather recently characterized Nup88 in its dynamic subcomplex with the oncogenic nucleoporin CAN/Nup214 seems to play several essential roles; depletion of the complex results in defective import-export processes, and eventual cell-cycle arrest;<sup>5</sup> and, in overexpressing cells, it seems that CAN/Nup214 and one of its interacting proteins, ie, Nup88, may function on both aspects of the nuclear pore complex.<sup>35</sup> Notably, preliminary experiments in our laboratory showed no increased expression of Can/Nup214 in our tumor samples thus suggesting an uncoupling of the latter from Nup88, at least in some instances. Interesting in this context is the fact that the Nup88 gene seems to be involved in therapy-related leukemias by translocation producing fusion proteins that may act as transcription factors modulating the expression of other genes.<sup>36</sup>

We can only speculate about the possible role(s) of the overexpressed Nup88 in malignant cells. A possible explanation is that its overexpression is simply the result of increased nucleocytoplasmic transport required to meet the increased demand of proteins by transformed cells. Increased traffic is indeed known to occur in this context, and it merits mention that the diameter of the pore channel seems to be increased in transformed mammalian cells.<sup>37</sup> An alternative explanation is that Nup88 may play a role in the formation and maintenance of annulate lamellae as outlined above: but, although this might explain their presence it would still not clarify their function. Irrespective of these speculations, our findings suggest that Nup88 may be a potentially significant marker given its dramatic overexpression in a broad spectrum of malignant tumors of most, if not all, major types. If these results were confirmed, Nup88 might be said to approach an ideal generic marker of transformation readily demonstrable in conventional tissue sections, and possibly also in cytological samples.

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