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Review

The Cancer Biomarker Osteopontin: Combination with Other Markers

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Abstract. *Osteopontin (OPN) is a biomarker for cancer progression and prognosis in multiple tumor types, however, it has not yet found entry into clinical diagnostics. In recent years, there has been an increasing recognition that marker combinations may have better diagnostic or prognostic potential than individual molecules. While various studies have analyzed OPN in conjunction with other markers, a comprehensive review of their published results is still lacking. OPN in tumor tissue or in the blood has been investigated in conjunction with a broad range of other markers in diverse types of cancer. OPN has been combined with cancer-specific markers, functionally converging biomolecules (angiogenesis, motility/adhesion, extracellular matrix, bone) and synergizing biomolecules (fibrinolytic system, calcium homeostatic proteins, squamous cell carcinoma antigen, NF-κB pathways, proteases). Clinical parameters of interest have been cancer detection, assessment of progression, and prognosis/treatment response. Some marker combinations with OPN are promising for detection, diagnosis or prognosis in various types of cancer. Yet, surprisingly, in many cases, the published results are conflicting, and no clear metrics have been developed for utilizing marker combinations in clinical decision making. With the current intense interest in multiplex marker panels, additional, large-scale studies are needed to realize the full diagnostic and prognostic potential of OPN.*

Osteopontin (OPN) has been extensively studied as a cancer biomarker, however, it has not yet found routine use in the clinic. We have recently performed a meta-analysis to evaluate the diagnostic and prognostic power of OPN. The results indicate that OPN is a cancer biomarker, and is related to stage, grade and early tumor progression in multiple cancer types. It is also a predictor of disease-free and overall survival in various malignancies (1, 2).

One aspect that has not been evaluated is the combination of OPN with other markers. Various benefits could conceivably be derived from such combinations. Markers that correlate with OPN and also measure cancer progression could enhance confidence. Markers for other aspects of transformation, such as growth rate, could refine the assessment. Furthermore, as elevated OPN levels are associated with about 30 types of cancer, the combination with cancer-type specific markers may aid in early detection. With the increasing use of multi-marker panels in clinical medicine, such combinations gain importance. This article reviews the literature previously evaluated for the meta-analyses for OPN plus other markers in cancer diagnosis and prognosis (Table I).

Cancer Type-Specific Markers

Breast cancer. As gains of function in estrogen receptor (ER)-related or EGF-related pathways drive the proliferation of most breast carcinomas, OPN has been measured together with biochemical markers for these pathways. Remarkably, there are conflicting results for the correlation of OPN with ER. OPN expression has been reported to be negatively correlated with ER and progesterone receptor (PR) (3), or not to correlate with the expression of ER (4). In another study, there was a significant positive association of carcinomas staining for OPN with staining for ERα, except for the subgroup of carcinomas staining positively for both S100A4 and for ERα (5). Mean OPN levels are higher in the triple-negative (ER^-, PR^-, $ERBB2^-$) breast cancer subtype than in the non-triple-negative subtype (6), which may reflect the abundance of the splice variant OPN-c (7). There are no correlations between OPN and estrogens. In patients without bone metastases, there are relationships between OPN and estrone sulfate only at baseline but not after anastrazole

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treatment (9). However, staining for OPN and ERα are independent predictors of outcome in breast cancer (8).

Breast cancer has a propensity to metastasize to bone. Therefore, OPN measurements in conjunction with various bone markers have been reported. Normal mammary epithelial cell have undetectable or weak immunoreactivity for osteonectin (OSN, SPARC) and OPN. In benign lesions, the expression of OPN is generally stronger than OSN. In breast carcinomas, there was no difference in the expression of OSN or OPN between ductal and lobular types. The levels of immunostaining for OSN or OPN correlated with increasing malignancy from normal breast tissue to carcinoma *in situ* to invasive carcinoma. The presence of microcalcifications was also associated with a high expression of OPN and OSN (10). Contrary to this, OPN and OSN were found not to correlate with lymph node involvement, tumor size, tumor grade, or expression of P53. The 5-year survival rates of OPN- and OSN-positive groups were not significantly different (5).

There are significant associations between *OPN* and osteoprotegerin (*OPG*) mRNA copy numbers in breast cancer, but not between these variables and histology or size of the primary tumors. The average *OPN* gene expression, but not the gene expression of *OPG*, significantly increases in ductal carcinoma *in situ* (DCIS) compared to normal breast tissue (11). Whereas there is no difference in baseline OPG and OPN serum values between patients with or without metastases, there are changes in their blood values in patients with progressive disease. Before breast cancer treatment, serum levels of OPG and OPN in patients do not correlate with each other. During 12 weeks of anastrazole treatment, patients with bone metastasis show a significant increase in both analytes, but there are no significant changes for patients without bone metastasis (9).

Bone sialoprotein (BSP) is expressed in 65% and OPN in 75% of breast carcinomas. There are *BSP* and *OPN* transcripts in both invasive and *in situ* carcinoma components, but not in surrounding stromal cells or in peritumoral macrophages (12). In breast cancer, the immunohistochemical staining pattern for BSP is frequently stronger in the cells lining the lumen of tumor glands but without the apical staining pattern for OPN. As in the case of OPN, infiltrating and poorly differentiated areas stain strongly for BSP (13). There is no correlation between OPN and BSP in breast cancer bone metastases, as OPN is more abundant in bone metastases than in primary tumors, which is not the case for BSP (13, 14).

The gene expression levels of bone morphogenic protein 2 (BMP2) and *OPN* are relatively low in normal breast tissue. Whereas the average *BMP2* mRNA level decreases over 12-fold in DCIS tissue, the average *OPN* gene expression increases in DCIS compared to normal breast tissue. *OPN* is overexpressed in 60% and *BMP2* is underexpressed in all DCIS samples. There are no significant correlations between *BMP2* or *OPN* mRNA copy numbers and the histology or size of the primary tumors. The average *BMP2* gene expression is approximately the same in bone metastatic tissue as in normal breast tissue (11).

In patients with bone metastasis, there is a weak correlation between serum levels of OPN and bone alkaline phosphatase (ALP), which is maintained during anastrozole treatment. In patients without bone metastases, there is a correlation between OPN and bone ALP only during treatment (9). OPN plasma levels in breast cancer are positively correlated with serum ALP, aspartate succinate aminotransaminase (AST) and absolute neutrophil count, while being negatively correlated with serum albumin, hemoglobin and total lymphocytes (15).

Extracellular matrix proteins are important in cancer metastasis as ligands for homing receptors and inducers of anti-anoikis signals. In normal breasts, the levels of collagen and *OPN* mRNA are low. Malignant tumors have increased α1 (I) pro-collagen and *OPN* mRNA expression compared with benign tissue. While collagen mRNA is present in over 90% of invasive breast carcinomas, *OPN* mRNA is displayed in about 75% at low to moderate levels, primarily in the tumor stroma surrounding the tumor nests. *OPN* mRNA exists in 60% of non-invasive DCIS that are positive for pro-collagen mRNA, with the expression of *OPN* mRNA generally being comparable to that of pro-collagen mRNA. Pro-collagen and *OPN* mRNA expression vary considerably between matched primary and secondary tumors (16). In breast cancer patients, there is a weak correlation between serum OPN, *C*-terminal peptide of type I collagen and cross-linked *N*-telopeptide of type I collagen (PINP), which is maintained during anastrozole treatment (9).

Breast tumor cells express OPN and thrombospondin 1 (TSP1). *TSP1* transcripts are essentially absent from normal or benign breast epithelia with no appreciable levels in stromal tissue components. However, TSP1 levels are raised in malignant epithelial tissue. TSP1 is elevated in all primary tumors and in all metastases over normal breast epithelia. In line with the OPN patterns of expression, there is no significant difference in TSP1 levels between primary tumors with and those without metastases, and between primary carcinomas and their associated metastases (17).

As an avidly calcium-binding protein, OPN has been assessed in conjunction with other regulators of calcium homeostasis. S100P is elevated in carcinoma that has spread to the axillary lymph nodes. There is a significant association of staining for S100P with several prognostic markers including S100A4, OPN, ERBB3, cathepsin D, pS2, and P53 (8). In minimally invasive breast cancer, positive immunocytochemical staining for S100A4, but not OPN, was found to correlate with the presence of higher vessel density in the primary tumor. There was a borderline association between staining for S100A4 and for OPN. Other variables, including tumor size, histologic grade, menopausal status, and age,

showed no significant association with positive staining for S100A4 or for OPN (5). Independent predictors of outcome are staining for S100A4 followed by that for OPN, involved lymph nodes, S100P, and ERα (8). While the survival times for patients with S100A4-negative or OPN-negative tumors are usually not significantly different, patients with both immunocytochemically detectable S100A4 and OPN in the primary tumors show a significant reduction in survival time over those with either one alone. However, although patients with OPN-negative tumors display a significant difference in survival between those stained positively or negatively for S100A4, the converse is not true. In minimally invasive breast cancer, lymph node status, tumor size, histologic grade, and vessel density improve the prediction of patient survival time over S100A4 or OPN alone (5).

Two major isoforms of calcitonin receptors result from alternative splicing of the primary mRNA transcript and differ by the inclusion or exclusion of a 16 amino acid insert within the first putative intracellular loop of the receptor. The insert-negative calcitonin receptor isoform of mRNA is expressed by all breast carcinomas. In contrast, only 40% of tumors express the insert-containing calcitonin receptor isoform. *OPN* mRNA is present in all of the tumor samples. Calcitonin and OPN are expressed in the tumor cells and not in the surrounding stroma (18).

Diverse variables have been associated with OPN in breast cancer. The gene expression levels of activin βA, inhibin α, OPN, and receptor activator of NF-κB ligand (RANKL) are relatively low in normal breast tissue. There are no significant correlations of activin βA, inhibin α, *OPN*, or *RANKL* mRNA copy numbers with histology or size of primary breast tumors. The average gene expressions of activin βA, inhibin α, and *RANKL* are no different in non-invasive and normal breast tissue. By contrast, the average *OPN* gene expression significantly increases in DCIS compared to normal breast tissue. Associations exist in more advanced tumors, where both activin βA and OPN are overexpressed in invasive tumor tissue and in bone metastatic tissue compared to normal breast tissue (11). Breast tumor cells express OPN and tyrosinase-related protein-1 (TYRP-1). TYRP1 is present in malignant cells but is at the limit of detection in normal and benign epithelia and stromal components, and it is lower than that of OPN. While *TYRP1* transcripts are detectable in malignant and non-malignant tissues, there are no significant quantitative differences between the types of tissue (17). Normal breasts have no urokinase plasminogen activation (uPA) and low levels of uPAR, PAI-1, and OPN. Non-invasive breast carcinomas contain low to moderate levels of uPA and variable levels of uPAR or PAI1, while *OPN* RNA is present in a fraction of non-invasive DCIS. There is no correlation between the development of bone metastases and the expression of the uPA system. *uPA* mRNA levels are comparable in primary tumors and their metastases, whereas *uPAR* mRNA is higher and *PAI1* mRNA lower in the bone metastases compared to their matching primaries (16).

Standard markers for the assessment of breast cancer include ER, PR, and HER2. The addition of OPN, especially the splice variant c, may add information on grade and stage and may identify the presence of triple-negative breast cancer. Most bone markers and extracellular matrix molecules tested seem to be less sensitive at detecting breasty cancer than OPN. Proteins of the S100 family can potentially contribute information on lymph node spread and vessel density.

Ovarian cancer. The primary biochemical marker for ovarian cancer is CA125. CA125 and OPN blood concentrations are elevated in ovarian cancer patients over patients with other gynecologic cancers, those with benign ovarian neoplasms, and disease-free females. Even in early stage cases, CA125 and OPN are indicative for ovarian cancer, with CA125 being the superior predictor. When either marker is elevated, the sensitivity for detecting ovarian cancer increases to almost 95%, and is higher compared to that of CA125 alone. However, the specificity decreases considerably. When both markers are elevated, the specificity increases, while the sensitivity decreases. While neither OPN nor CA125 is affected by the histological type of ovarian cancer, plasma OPN, but not plasma CA125, varies by International Federation of Gynecology and Obstetrics (FIGO) stage and is correlated with the volume of ascites (19-21). In a multi-analyte blood test for serous ovarian carcinoma, multiple markers perform significantly better than single markers, with little difference between 4- and 5-marker combinations. CA125, OPN and matrix metalloproteinase 7 (MMP7) are diagnostic. OPN increases the CA125-based accuracy to 98%. A classifier based on CA125, OPN, and kallikrein-related peptidase 10 (KLK10) reaches over 95% sensitivity and specificity of almost 100%. For a wide range of classifier configurations, the performance of individual markers decreases in the order of OPN, CA125, MMP7, KLK10, and secretory leukocyte protease inhibitor (SLPI) (22). In a separate study, CA125, OPN, CA 19-9, carcinoembryonic antigen (CEA), chondrocyte protein YKL40, ERBB2, a disintegrin and metalloproteinase domain 8 (ADAM8), SLPI, vascular endothelial growth factor (VEGF), insulin-like growth factor-2 (IGF2), and connective tissue growth factor (CTGF) were combined into a panel assay. However, no improvements for ovarian cancer diagnosis using a multi-marker approach were identified (23). All CA125-deficient ovarian carcinomas express kallikrein 10 (HK10), kallikrein 6 (HK6), OPN, and claudin-3 (CLDN3) at some level. Breast cancer-associated DF3 antigen (DF3) expression is present in 95% of CA125-deficient cancers and identifies a larger fraction of tumors than OPN or CLDN3 (an integral membrane protein important in tight junctions). The intensity of the staining pattern is similar for HK10 and HK6, and this is stronger than for OPN, CLDN3, and DF3 (24). Mean CA125

Table I. *OPN plus other markers with references that provide the basis for this review.*

Cancer	Biomarkers	Characterization	n	Specimen	Technique	Description	Reference
Ampullary neoplasm	Osteonectin	Cancer	50	Tumor	ISH	Path score	59
		Chronic pancreatitis	12	Tissue	ISH	Path score	
		Normal pancreas	17	Tissue	ISH	Path score	
Bone cancer	*VEGF*	Osteosarcoma	57	Tumor	IHC	Path score	97
		Osteoblastoma	11	Tumor	IHC	Path score	
		Normal (remodeling areas)	5	Tissue	IHC	Path score	
Bone cancer	*IHH, FGFR3, PTHR1, PCNA, osteocalcin, bone sialoprotein*	Osteochondroma (on the basis of multiple hereditary exostoses)	8	Tumor	IHC	Path score	102
		Normal cartilage	4	Tissue	IHC	Path score	
Bone cancer	*Osteocalcin, osteonectin, VS38c, S-100, CD34, collagen I, vimentin, cytokeratins*	Osteosarcoma of the mandible	1	Tumor	IHC	Path score	98
Bone cancer	*THBS3, SPARC*	Non-metastatic osteosarcoma	3	Tumor	Microarray	Relative abundance	100
		Metastatic osteosarcoma, primary Tumor	3	Tumor	Microarray	Relative abundance	
		Osteosarcoma, metastasis	3	Tissue	Microarray	Relative abundance	
Bone cancer	*Vimentin, VEGF, CD10, alkaline phosphatase, keratin*	Osteosarcoma of the breast	1	Tumor	IHC		101
Bone cancer	*Osteocalcin, osteonectin, type I collagen*	Normal	29	Blood	RT-PCR (semiquantitative)	Relative abundance	99
Bone cancer	*Runx2, Notch1, Delta*	Osteosarcoma	10	Tumor	IHC	Path score	110
Breast cancer	*Osteonectin*	Normal breast	21	Tissue	IHC	Path score	10
		Benign growth	28	Tumor	IHC	Path score	
		Carcinoma *in situ* (early stage primary tumor)	25	Tumor	IHC	Path score	
		Lymph node-negative primary tumor	11	Tumor	IHC	Path score	
		Lymph node-positive primary tumor	15	Tumor	IHC	Path score	
Breast cancer	*Bone sialoprotein*	Primary tumor	12	Tumor	IHC	Path score	14
		Bone metastasis	12	Bone	IHC	Path score	
Breast cancer	*uPA, uPAR-1, PAI-1, and collagen*	Normal breast	5	Tissue	ISH (RNA)	Path score	16
		DCIS (early stage primary tumor)	13	Tumor	ISH (RNA)	Path score	
		Invasive breast cancer	23	Tumor	ISH (RNA)	Path score	
		Bone metastasis	25	Bone	ISH (RNA)	Path score	
Breast cancer	*Calcitonin receptor and BSP*	Breast cancer	18	Tumor	RT-PCR	Positive/negative	18
Breast cancer	*BSP*	Bone metastasis	10	Bone	IHC	Path score	13
		Bone metastasis	10	Bone	ISH (RNA)	Path score	
Breast cancer	*Osteonectin*	Lymph node-negative primary tumor	113	Tumor	IHC	Path score	4
		Lymph node-positive primary tumor	140	Tumor	IHC	Path score	
Breast cancer	*Osteoprotegerin, BGP, BAP, ICTP, NTx, E2, E1, and E1-S*	Baseline	34	Serum	ELISA	µg/l	9
		2 Weeks' anastrozole treatment	34	Serum	ELISA	µg/l	
		4 Weeks' anastrozole treatment	34	Serum	ELISA	µg/l	
		8 Weeks' anastrozole treatment	34	Serum	ELISA	µg/l	
		16 Weeks' anastrozole treatment	34	Serum	ELISA	µg/l	
Breast cancer	*HER2, ER, PR*	Triple-negative breast cancer	56	Tumor	IHC	Path score	7
Breast cancer	*ER, PR, HER2*	Lymph node-negative primary tumor	10	Tumor	RT-PCR (real-time)	Relative abundance	11
		Lymph node-positive primary tumor	14	Tumor	RT-PCR (real-time)	Relative abundance	
Breast cancer	*Albumin, AST, alkaline phosphatase*	Breast cancer with metastasis	70	Plasma	ELISA	µg/l	15
		Breast cancer, 6 months after treatment	44	Plasma	ELISA	µg/l	
		Normal	35	Plasma	ELISA	µg/l	
Breast cancer	*S100P*	Invasive breast cancer	297	Tumor	IHC	Path score	8
Breast cancer	*HER2, ER, PR*	Triple-negative primary breast cancer	22	Tumor	IHC	Path score	6
		Non-triple-negative primary breast cancer	95	Tumor	IHC	Path score	
Breast cancer	*TYRP-1, TSP-1*	Primary tumor with metastases	22	Tumor	RT-PCR (real-time)	Relative abundance	17
		Primary tumor without metastases	9	Tumor	RT-PCR (real-time)	Relative abundance	

Table I. *continued*

Table I. *continued*

Cancer	Biomarkers	Characterization	n	Specimen	Technique	Description	Reference
		Lymph node metastasis	22	Tumor	RT-PCR (real-time)	Relative abundance	
		Fibroadenoma	10	Tumor	RT-PCR (real-time)	Relative abundance	
		Normal	5	Tissue	RT-PCR (real-time)	Relative abundance	
Breast cancer	*LH, progesterone*	Premenopausal, healthy	21	Plasma	ELISA	ng/ml	111
		Postmenopausal, healthy	14	Plasma	ELISA	ng/ml	
Breast cancer	*S100A4, ER*	Grade 1, 2 breast cancer	203	Tumor	IHC	Path score	5
		Grade 3 breast cancer	65	Tumor	IHC	Path score	
Breast cancer	*ER, PR, p53, ErbB2, ErbB3, Ki67, Bag1, Bcl2, CHK2, Cyclin D1, hTERT, p16, p21, p27, Survivin*	Grade I (Bloom & Richardson)	32	Tumor	IHC	Path score	3
		Grade II (Bloom & Richardson)	39	Tumor	IHC	Path score	
		Grade III (Bloom & Richardson)	29	Tumor	IHC	Path score	
Breast cancer	*BSP*	Invasive breast carcinoma	15	Tumor	ISH (RNA)	Path score	12
Cervical cancer	*SCC*	Primary tumor, keratinizing	55	Plasma	ELISA	ng/ml	32
		Primary tumor, non-keratinizing	17	Plasma	ELISA	ng/ml	
		Primary tumor, non-squamous	9	Plasma	ELISA	ng/ml	
Cervical cancer	*HPV*	Squamous cell carcinoma	29	Tumor	Microarray	Relative abundance	33
		Normal	18	Tissue	Microarray	Relative abundance	
Colorectal cancer	*ANG-2 and Tie-2*	Cancer	1	Tumor	RT-PCR (real-time)	Relative abundance	53
		Normal	1	Tissue	RT-PCR (real-time)	Relative abundance	
Colorectal cancer	*Osteonectin*	Tumor progression		Tumor	Microarray		54
Endometrial cancer	*Ezrin*	Grade 1endometrioid carcinoma	126	Tumor	IHC	Path score	31
		Grade 2 endometrioid carcinoma	28	Tumor	IHC	Path score	
		Grade 3 endometrioid carcinoma	10	Tumor	IHC	Path score	
Endometrial cancer	*CEACAM-1*	Endometrial hyperplasia	17	Tumor	IHC	Path score	30
		Normal	20	Tissue	IHC	Path score	
Esophageal cancer	*Cathepsin, RAS, p53*	Stage 2 adenocarcinoma	7	Tumor	IHC	Path score	47
		Stage 3 adenocarcinoma	12	Tumor	IHC	Path score	
		Stage 4 adenocarcinoma	1	Tumor	IHC	Path score	
Esophageal cancer	*SCC, Cyfra, CEA*	No lymph node metastasis (N0) Squamous cell carcinoma	47	Plasma	ELISA	ng/ml	45
		Lymph node metastasis (N1) Squamous cell carcinoma	56	Plasma	ELISA	ng/ml	
Esophageal cancer	*RhoC*	Squamous cell carcinoma	80	Tumor	IHC		46
		Gastric cancer					
Gastric cancer	*Pepsinogen*	Gastric cancer	144	Serum	ELISA	ng/ml	48
		Dysplasia	60	Serum	ELISA	ng/ml	
		Atrophic gastritis	113	Serum	ELISA	ng/ml	
		Ulcer	70	Serum	ELISA	ng/ml	
		Superficial gastritis	92	Serum	ELISA	ng/ml	
		Normal	91	Serum	ELISA	ng/ml	
Gastric cancer	*NF-kB, MMP*	Gastric cancer without LN metastasis	30	Tumor	IHC	Path score	49
		Gastric cancer with LN metastasis	26	Tumor	IHC	Path score	
		Gastric cancer with distant and LN metastasis tissue	16	Tumor	IHC	Path score	
		Normal surrounding	12	Tissue	IHC	Path score	
Gastric cancer	*COX-2, VEGF, CD34*	Cancer	53	Tumor	IHC	Path score	50
		Normal	40	Tissue	IHC	Path score	
Gastric cancer	*BMP, osteonectin*	Gastric carcinoid tumor	1	Tumor	IHC	Path score	51
Glioma	*SPOCK-1*	Glioblastoma		Tumor	RNA (SSH)	Relative abundance	89
		Pilocytic astrocytoma		Tumor	RNA (SSH)	Relative abundance	
Glioma	*MCP*	Grade III anaplastic astrocytoma/ Oligodendroglioma	1	Tumor	RNA (SAGE)	Relative abundance	90
		Grade II well-differentiated astrocytoma/oligodendroglioma		Tumor	RNA (SAGE)	Relative abundance	
Glioma	*CA9, erythropoietin,* VEGF, HIF-1α	Glioblastoma	15	Tumor	RT-PCR (semiquantitative)		87

Table I. *continued*

Table I. *continued*

Cancer	Biomarkers	Characterization	n	Specimen	Technique	Description	Reference
		Low-grade astrocytoma (WHO grade 2)	15	Tumor	RT-PCR (semiquantitative)		
		Normal brain	3	Tissue	RT-PCR (semiquantitative)		
Glioma	*VEGF, integrin αVβ3*	Glioblastoma	23	Tumor	IHC	Path score	88
		Anaplastic astrocytoma	13	Tumor	IHC	Path score	
		Low-grade astrocytoma	30	Tumor	IHC	Path score	
Granular cell tumor	*KP-1*	Granular cell tumor	20	Tumor	IHC	Path score	103
		Neurilemmoma	12	Tumor	IHC	Path score	
		Amputation neuroma	3	Tumor	IHC	Path score	
		Malignant peripheral nerve sheath tumor	3	Tumor	IHC	Path score	
		Granular cell epulis	2	Tissue	IHC	Path score	
Head and neck cancer	*HIF-1, systemic VEGF, CAIX*	Median hemoglobin >12.8 g/dl	17	Tumor	IHC	Path score	82
		Median hemoglobin ≤12.8 g/dl	17	Tumor	IHC	Path score	
Head and neck cancer	*HIF-1, CAIX*	Survival		Tumor	IHC	Path score	82
Head and neck cancer	*SCC*	Cancer	37	Serum	ELISA	ng/ml	86
		Normal	16	Serum	ELISA	ng/ml	
Head and neck cancer	*CAIX*	SCC, tumor oxygenation	84	Tumor	IHC	Path score	80
Head and neck cancer	*HIF-1α, CA9*	Proportion of tumor pO_2 <2.5 mm Hg		Tumor	IHC	Path score	81
		Median pO_2		Tumor	IHC	Path score	
		Hemoglobin (g/dl)		Tumor	IHC	Path score	
Head and neck cancer	*CD44v6*	Mild laryngeal intraepithelial neoplasia	21	Tumor	IHC	Path score	83
		Moderate laryngeal intraepithelial neoplasia	8	Tumor	IHC	Path score	
		Severe laryngeal intraepithelial neoplasia	53	Tumor	IHC	Path score	
Head and neck cancer	*MMP1, MMP12, NTS*	Head and neck squamous cell carcinoma *vs*. normal surrounding	21	Tumor	Microarray	Relative abundance	84
Head and neck cancer	*CTGF*	Laryngeal squamous cell carcinoma	41	Tumor	IHC		85
		Paracancerous tissue (surrounding normal)	20	Tissue	IHC		
Head and neck cancer	*CTGF*	Laryngeal squamous cell carcinoma	41	Tumor	IHC		85
		Paracancerous tissue (surrounding normal)	20	Tissue	IHC		
Liver cancer	*E-Cadherin*	Recurrence within 12 months	6	Tumor	RT-PCR (real-time)	Relative abundance	68
		No recurrence within 12 months	38	Tumor	RT-PCR (real-time)	Relative abundance	
Liver cancer	*AFP, PIVKA II*	Hepatocellular carcinoma	62	Plasma	ELISA	ng/ml	65
		Chronic liver disease	60	Plasma	ELISA	ng/ml	
		Normal	60	Plasma	ELISA	ng/ml	
Liver cancer	*TATI*	Hepatocellular carcinoma	235	Tumor	RT-PCR (semi-quantitative)	Overexpression	66
Liver cancer	*Glypican 3, Spondin-2, PEG10, EDIL3*	Hepatocellular carcinoma	37	Tumor	Microarray	Relative abundance	70
		Childhood hepatoblastoma	7	Tumor	Microarray	Relative abundance	
		HCC adjacent normal	32	Tissue	Microarray	Relative abundance	
		Normal liver	21	Tissue	Microarray	Relative abundance	
Liver cancer	*AFP, P53*	Hepatocellular carcinoma	240	Tumor	RT-PCR (semi-quantitative)	OPN/S26 ratio	60
		Non-HCC livers	271	Tissue	RT-PCR (semi-quantitative)	OPN/S26 ratio	
Liver cancer	*AFP*	Lymph node metastasis	9	Tumor	IHC	Path score	61
		No lymph node metastasis	63	Tumor	IHC	Path score	
Liver cancer	*Stathmin, P53*	HCC	156	Tumor	RT-RCR (semi-quantitative)	OPN/S26 ratio	69
Liver cancer	*ICAM-1*	HCC with recurrence		Plasma	ELISA		67
		HCC without recurrence		Plasma	ELISA		
		Overall survival		Plasma	ELISA		
		Disease-free survival		Plasma	ELISA		
Liver cancer	APRI	HBV-infected liver	39	Plasma	ELISA	ng/ml	112

Table I. *continued*

Table I. *continued*

Cancer	Biomarkers	Characterization	n	Specimen	Technique	Description	Reference
		HBV-infected liver with cirrhosis	30	Plasma	ELISA	ng/ml	
		HBV-infected liver with HCC	11	Plasma	ELISA	ng/ml	
		healthy liver	14	Plasma	ELISA	ng/ml	
Liver cancer	*E-Cadherin, β-catenin*	HCC, Edmondson-Stenier grade I	8	Tumor	IHC	Path score	64
		HCC, Edmondson-Stenier grade II	88	Tumor	IHC	Path score	
		HCC, Edmondson-Stenier grade III-IV	29	Tumor	IHC	Path score	
Liver cancer	*Annexin A10, α-Fetoprotein*	Unifocal primary HCC	294	Tumor	IHC	Path score	62
Liver cancer	*CD44, AFP*	Hepatocellular carcinoma	302	Tumor	IHC	Path score	63
Gestational trophoblastic disease	*CEACAM-1*	Hydatiform mole	21	Tumor	IHC	Path score	30
		Choriocarcinoma	6	Tumor	IHC	Path score	
Lung cancer	*ALP*	Stage I NSCLC	32	Plasma	ELISA	ng/ml	72
		Stage II NSCLC	8	Plasma	ELISA	ng/ml	
		Stage III NSCLC	43	Plasma	ELISA	ng/ml	
		Stage IV NSCLC	47	Plasma	ELISA	ng/ml	
Lung cancer	*NF-κB p65, p-AKT, p-ERK, p-STAT-3, MMP-1, MMP-2, MMP-9*	Proliferative rate of adenocarcinoma		Tumor	IHC	Path score	75
Lung cancer	*CAIX, CD44*	2-Year overall survival	20	Plasma	ELISA	pg/ml	71
Lung cancer	*osteonectin*	NSCLC	82	Tumor	RT-PCR (real-time)	Relative abundance	74
Lung cancer		Normal surrounding	82	Tissue	RT-PCR (real-time)	Relative abundance	
Lung cancer	*VEGF, CD34*	Adenocarcinoma, stage I NSCLC	55	Tumor	IHC	Path score	77
		Squamous cell carcinoma, stage I NSCLC	32	Tumor	IHC	Path score	
Lung cancer	*RAS*	NSCLC	40	Tumor	IHC	Path score	76
		Squamous cell carcinoma	16	Tumor	IHC	Path score	
		adenocarcinoma, stage I NSCLC	24	Tumor	IHC	Path score	
		Small cell carcinoma	18	Tumor	IHC	Path score	
Lung cancer	*PAI-1, VEGF*	Non-small cell lung cancer	156	Plasma	ELISA	ng/ml	73
Lung cancer	*MPF, Mesothelin*	lung cancer	10	Serum	ELISA	ng/ml	78
Melanoma	*N-Cadherin, Osteonectin, PKCα, Glypican-3*	Metastatic disease	102	Tumor	IHC	Path score	91
Melanoma	*HIF-1α, CAIX, TNF-α, Apaf-1, αvβ3 integrin, CD44/HCAM*	Melanoma	148	Tumor	IHC	Path score	92
		Naevus	31	Tumor	IHC	Path score	
Non-melanoma skin cancer	*β-Catenin*	Basal cell carcinoma with matrical differentiation	2	Tumor	IHC	Path score	93
Mesothelioma	*Mesothelin*	Malignant pleural mesothelioma	94	Serum	ELISA	ng/ml	79
		Pleural metastases of various carcinomas	41	Serum	ELISA	ng/ml	
		Benign pleural lesions associated with asbestos exposure	32	Serum	ELISA	ng/ml	
		Asbestos-exposed healthy subjects	112	Serum	ELISA	ng/ml	
Mesothelioma	*MPF, Mesothelin*	Malignant mesothelioma	66	Serum	ELISA	ng/ml	78
		Benign disease	21	Serum	ELISA	ng/ml	
		Normal	20	Serum	ELISA	ng/ml	
Myeloma	*MIP-1α, carboxy-terminal telopeptide of collagen I, collagen I propeptide, deoxypyridinoline, bone-specific alkaline phosphatase*	Myeloma with bone involvement	22	Plasma	ELISA	ng/ml	106
		Normal	22	Plasma	ELISA	ng/ml	
Myeloma	*Creatinine, b2 microglobulin, albumin, serum M-protein concentration, serum LDH, CRP, HGF*	Myeloma	114	Serum	ELISA	ng/ml	107
		Normal	30	Serum	ELISA	ng/ml	
Myeloma	*IL-6, IL-11, IL-10,* TNF-α, sIL-6R, IL-11,	Cancer, bone marrow stromal cells in culture	16	Supernatant	ELISA	ng/ml	113

Table I. *continued*

Table I. *continued*

Cancer	Biomarkers	Characterization	n	Specimen	Technique	Description	Reference
	IL-10, TNF-α, BAFF, HGF	Normal, bone marrow stromal cells in culture	11	supernatant	ELISA	ng/ml	
Oral cancer	*BSP, DMP1, MMP-2, MMP-3, MMP-9*	Oral squamous cell carcinoma	87	Tumor	IHC	Path score	42
Oral cancer	*Osteonectin, collagen IV*	Salivary pleomorphic adenoma	43	Tumor	IHC	Path score	44
Oral cancer	*Cytokeratins, vimentin, desmin, S-100, αSMA, BMPs, Ki-67*	Spindle cell carcinoma (highly malignant SCC)	1	Tumor	IHC	Path score	43
Ovarian cancer	*CA125*	Cancer	234	Serum	ELISA	ng/ml	25
		Normal	38	Serum	ELISA	ng/ml	
Ovarian cancer	*ADAM-8, CA-125, CA 19-9, carboxypeptidase A1, CEA, connective tissue growth factor, EGFR, E-CAM, Her2, galectin-1, IGF-2, IL-1, IL-7, mesothelin, MIF, secretory leukocyte peptidase inhibitor, TNF, VEGF, chitinase 3–like 1*	Ovarian cancer	19	Plasma	Multiplexed proximity ligation assay	Relative abundance	23
		Normal	20	Plasma	Multiplexed Proximity Ligation Assay	Relative abundance	
Ovarian cancer	*HNF-1β*	Clear cell carcinoma	30	Tumor	IHC	Path score	28
		Serous adenocarcinoma	30	Tumor	IHC	Path score	
Ovarian cancer	*CD68, osteonectin, osteocalcin*	Serous papillary cystadenocarcinoma	15	Tumor	ISH	Path score	29
Ovarian cancer	*SLPI, MMP-7, KLK10, CA125*	Serous papillary carcinoma	67	Tumor	Microarray	Relative abundance	22
		Normal	8	Tissue	Microarray	Relative abundance	
Ovarian cancer	*Prostasin*	Ovarian cancer cells in culture	3	Cells	Microarray	Relative abundance	115
		Human ovarian surface epithelial cells in culture	3	Cells	Microarray	Relative abundance	
Ovarian cancer	*CA125, SMRP, HE4, CA72-4, activin, inhibin, EGFR, ERBB2*	Cancer	67	Serum	ELISA	ng/ml	27
		Benign disease	166	Serum	ELISA	ng/ml	
Ovarian cancer	*CA125, IGF II, leptin, prolactin*	Cancer	15	Serum			20
		Benign neoplasm	33	Serum			
		Normal	21	Serum			
Ovarian cancer	*CA125*	Cancer	32	Plasma	ELISA	ng/ml	21
		Benign tumor	34	Plasma	ELISA	ng/ml	
		Other gynecologic cancer (12 uterine cervical, 13 endometrial, 3 vulvar, 2 tubal)	30	Plasma	ELISA	ng/ml	
		Normal	31	Plasma	ELISA	ng/ml	
Ovarian cancer	*p53, MIB1, BCL2, WT1, HER-2/neu, C-KIT, survivin*	Low-grade carcinoma	22	Tumor	IHC	Path score	115
		High-grade carcinoma	47	Tumor	IHC	Path score	
Ovarian cancer	*CA125, KLK10, KLK6, claudin 3, DF3, VEGF, MUC1, mesothelin, HE4, CA19-9*	CA125-deficient ovarian cancer	60	Tumor	IHC	Path score	24
Ovarian cancer	*CA125*	Cancer, preoperative	38	Plasma	ELISA	ng/ml	26
		Cancer postoperative	38	Plasma	ELISA	ng/ml	
Ovarian cancer	*Leptin, prolactin, IGF2, MIF, CA-125*	Cancer	156	Serum	Multiplexed Proximity Ligation assay	pg/ml	19
		Normal	362	Serum	Multiplexed proximity ligation assay	pg/ml	

Table I. *continued*

Table I. *continued*

Cancer	Biomarkers	Characterization	n	Specimen	Technique	Description	Reference
Pancreatic cancer	*ADAM-8, CA-125, CA 19-9, carboxypeptidase A1, CEA, connective tissue growth factor, EGFR, E-CAM, Her2, galectin-1, IGF2, IL-1, IL-7, mesothelin, MIF, secretory leukocyte peptidase inhibitor, TNF, VEGF, chitinase 3–like 1*	Cancer	18	Plasma	Multiplexed Proximity Ligation Assay	Relative abundance	23
		Normal	19	Plasma	Multiplexed Proximity Ligation Assay	Relative abundance	
Pancreatic cancer	*MIC-1, CA19-9, HIP, TIMP-1*	Cancer	50	Serum	ELISA	ng/ml	55
		Chronic pancreatitis	50	Serum	ELISA	ng/ml	
		Normal	50	Serum	ELISA	ng/ml	
Pancreatic cancer	*HER2, K-RAS, P53, E-cadherin, VEGF-C, podoplanin, mismatch repair genes*	Osteoclast-like giant cell tumor in mucinous cystadenocarcinoma	1	Tumor	IHC	Path score	57
Pancreatic cancer	*E-Cadherin, β-catenin*	Ductal adenocarcinoma	15	Tumor	IHC	Path score	58
		Undifferentiated pancreatic carcinoma	10	Tumor	IHC	Path score	
Pancreatic cancer	*CK7, MUC1, CA 19-9, CEA, p53, MUC2, chromogranin A*	Pancreatic ductal adenocarcinoma associated with diffuse psammomatous calcification	1	Tumor	IHC	Path score	56
Pilomatricoma	*Gla protein, Osteonectin*	Benign tumor	6	Tumor	RNA (Northern blot)	Positive/negative	116
		Normal	1	Tissue	RNA (Northern blot)	Positive/negative	
Prostate cancer	*PSA, ALP, total acid phosphatase, prostatic acid phosphatase, LDH, creatinine, albumin, AST*	Hormone-refractory prostate carcinoma	100	Plasma	ELISA	ng/ml	36
Prostate cancer	*Carboxyterminal-telopeptide of type I collagen, aminoterminal-propeptide of type I procollagen, bone specific ALP, PSA*	Metastatic prostate cancer (M1)	28	Plasma	ELISA	µg/l	35
		Prostate cancer with LN involvement (N1M0)	30	Plasma	ELISA	µg/l	
		Non-metastatic prostate cancer (N0M0)	32	Plasma	ELISA	µg/l	
		Benign prostate hyperplasia	35	Plasma	ELISA	µg/l	
		Normal	29	Plasma	ELISA	µg/l	
Prostate cancer	*PSA*	Cancer	47	Tumor	IHC	Path score	34
		Benign prostate hyperplasia	12	Tumor	IHC	Path score	
Prostate cancer	*VEGF, HIF-1α*	Radiotherapy	201	Tumor	IHC	Path score	38
		Radical prostatectomy	285	Tumor	IHC	Path score	
Prostate cancer	*MMP-2, MMP-9, TIMP-1, PGE2, COX-2, PSA*	Prostate cancer	96	Plasma	ELISA	ng/ml	38
		Benign prostate hypertrophy	92	Plasma	ELISA	ng/ml	
		Normal	125	Plasma	ELISA	ng/ml	
Renal cancer	*CD44s, CD44v6, Ki-67*	Papillary renal cell cancer	43	Tumor	IHC	Path score Path score	117
Renal cancer	*Ki-67*	Fuhrman nuclear grade 1	29	Tumor	IHC	Path score	39
		Furhrman nuclear grade 2	69	Tumor	IHC	Path score	

Table I. *continued*

Table I. *continued*

Cancer	Biomarkers	Characterization	n	Specimen	Technique	Description	Reference
		Fuhrman nuclear grade 3	45	Tumor	IHC	Path score	
		Fuhrman nuclear grade 4	28	Tumor	IHC	Path score	
Renal cancer	*LOT1, HSP90, CSNK1, thymosin b4, Serum amyloid A, ceruloplasmin, SPARC, Cap43*	Renal cell carcinoma	1	Tumor	RNA (reverse Northern dot blot)	Densitometry	41
Renal cancer	*Carboxyterminal-telopeptide of type-I collagen (ICTP), bone bALP, ALAT, GGT, Albumin, LDH*	Renal cell carcinoma, non-bone metastases	20	Plasma	ELISA	µg/l	35
		Renal cell carcinoma, bone metastases	17	Plasma	ELISA	µg/l	
		Renal cell carcinoma, regional lymph node metastases	11	Plasma	ELISA	µg/l	
		Renal cell carcinoma, no metastases	32	Plasma	ELISA	µg/l	
		Normal	52	Plasma	ELISA	µg/l	
Thyroid cancer	*CD44v6*	Papillary thyroid cancer	71	Tumor	IHC	Path score	95
		Microfollicular adenoma	4	Tumor	IHC	Path score	
		Macrofollicular adenoma	3	Tumor	IHC	Path score	
		Multinodular goiter	5	Tumor	IHC	Path score	
		Normal	34	Tissue	IHC	Path score	
Thyroid cancer	*BSP, ON, OC, MGP, BMP-2, BMP-4*	Papillary carcinoma	6	Tumor	RNA (Northern blot)	Positive/negative	96
		Normal	1	Tissue	RNA (Northern blot)	Positive/negative	
Thyroid cancer	*CST6, CXCL14, DHRS3*	Papillary thyroid carcinoma	28	Tumor	RT-PCR (real-time)	Relative abundance	94
		Follicular thyroid carcinoma	17	Tumor	RT-PCR (real-time)	Relative abundance	
		Follicular thyroid adenoma	12	Tumor	RT-PCR (real-time)	Relative abundance	
		Normal	14	Tissue	RT-PCR (real-time)	Relative abundance	
Soft tissue sarcoma	*Cytokeratin, vimentin, desmin, actin, FVIII, CD34, CD31, CD44, UEA-1, MIB1*	Primary sarcomas of major blood vessels	7	Tumor	IHC	Path score	105
Soft tissue sarcoma	*Met, HGF*	Soft tissue tumor	15	Tumor	RT-PCR	Densitometry	104
		Normal surrounding	15	Tissue	RT-PCR	Densitometry	

levels correlate with active recurrent epithelial ovarian cancer as compared to disease in remission. OPN displays the same trends, with lower statistical significance. Among patients who have undergone primary cytoreductive surgery, OPN levels decrease postoperatively in over 75% of all cases, compared with 90% decreases in CA125 and 95% decreases in either OPN or CA125. Patients with a complete response over 4-9 cycles of chemotherapy treatment after debulking surgery have a consistent downward trend in both OPN and CA125 values. However, the longitudinal course of OPN is inferior to CA125 in predicting complete clinical responders *versus* partial/non-responders. Those patients who do not achieve a complete response uniformly show an initial increase in OPN levels followed by a decrease, while non-responders have final OPN values higher than their pretreatment values. By contrast, the CA125 values in this group decrease in all subsequent serum samples in a pattern indistinguishable from those in the complete response group. Overall, rising or falling OPN levels correlate with progression or regression of disease in 70% of patients *versus* 90% of CA125 measurements simultaneously performed. The combination is more accurate than OPN alone, but not superior to CA125 (25, 26). In patients with a pelvic mass, comparison of the post-menopausal benign group to the pre-menopausal benign group shows a statistical difference between the median levels of the serum markers CA125, human epididymis protein 4 (HE4), OPN, and inhibin. However, OPN and inhibin cannot increase the detection sensitivity for the combination of HE4 and CA125 (27).

All clear cell carcinomas of the ovary with a high OPN immunohistochemistry score also show a high hepatocyte nuclear factor-1β (HNF-1β) score. The frequency of OPN-positive cells is equal to or less than that of HNF-1β-positive cells. There are no cases that show a combination of high OPN and low HNF-1β scores. (28) Serum from patients with

ovarian cancer has elevated levels of macrophage migration inhibitory factor (MIF), prolactin, and OPN, as well as low levels of leptin and IGF2 compared with healthy females. In early stage cases, only IGF2 and OPN are indicative of ovarian cancer. The concentrations of IGF2, leptin and prolactin do not allow the distinction between females with ovarian cancer and those with benign ovarian disease (19, 20). In ovarian serous papillary cystadenocarcinoma tissues, psammoma bodies stain positively for OPN but negatively for osteocalcin (OC) and OSN. *OPN* mRNA-positive cells belong to areas of tumor stroma. CD68 is positively stained in those cells expressing *OPN* mRNA (29).

The published results suggest that in ovarian cancer the combination of CA125 with OPN and DF3 may gain sensitivity (due to DF3) and information on stage and presence of ascites (due to OPN). The addition of IGF2 to a marker panel could also be beneficial for sensitive detection.

Endometrial cancer. In endometrial carcinomas, OPN has been studied in conjunction with the adhesion receptor carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) and the motility-signaling molecule ezrin, which is a member of the ezrin/radixin/moiesin (ERM) complex. Both OPN and CEACAM1 are expressed in endometrial cancer, and both interact with integrin β3. CEACAM1 is co-expressed with OPN in normal endometrium and in endometrial neoplasia, implying the possibility that the two molecules may form a functional complex (30). A significant correlation exists between strong ezrin expression and primary tumor stage as well as overall survival. However, there is no correlation between ezrin expression and OPN expression. In patients with strongly ezrin-expressing tumors, the 5-year survival rate was lower. In contrast, patients with strong OPN expression a tendency toward more favorable outcome was observed (31).

Cervical cancer. There is a correlation between OPN and Squamous cell carcinoma (SCC) antigen levels in cervical cancer cases. For the detection of cervical cancer, the sensitivity and specificity of plasma OPN are better than SCC. There is a significant increase in sensitivity when combining OPN with SCC *versus* SCC alone (32). In both cervical cancer cases infected with HPV-16 and those infected with non-HPV-16 HPVs, the expression of 36 genes was found to be altered more than 3-fold. However, 23 genes, mainly related to DNA replication and protein modification, are specifically associated with cancers infected by HPV-16. Five genes, mainly involved in cell growth and apoptosis, are associated with cancers infected by non-HPV-16 HPV. The genes for OPN, VEGF, cell cycle controller 2 (CDC2), and CDC2-associate protein (CKS2) are coordinately differentially regulated between cancer and normal tissue. These genes encode for proteins that are part of a signaling pathway associated with tumor cell DNA fragmentation, cell survival and apoptosis as well as regulation of signal transduction (33).

Prostate cancer. Prostate-specific antigen (PSA) is the commonly used clinical marker for prostate cancer diagnosis and prognosis. Among healthy men, benign prostate hyperplasia patients, prostate cancer patients with lymph node-negative and lymph node-positive subgroups, PSA significantly correlates with OPN (34-36). However, one study was unable to confirm this correlation (37). Variables that are predictors of survival in hormone-refractory prostate carcinoma include OPN and PSA (36).

The propensity of prostate cancer to metastasize to bone has prompted investigations into bone markers as disease indicators. About 90% of prostate tumors stain strongly for BSP. The immunohistochemical staining pattern for BSP is frequently strong in the cells lining the lumen of tumor glands but without the apical staining pattern for OPN. Infiltrating and poorly differentiated areas stain strongly for BSP and OPN. Whereas OPN is higher in primary tumors than in bone metastases, BSP is more abundant in metastases than in primary tumors (14). The concentrations of various bone markers (OPN, carboxyl-terminal telopeptide of type I collagen (ICTP), bone-specific ALP, and PINP) do not differ among healthy individuals, men with benign prostate hyperplasia, prostate cancer patients with lymph node-negative and lymph node-positive subgroups, except for OPN where benign prostate hyperplasia patients show a higher concentration than controls. All four markers are significantly elevated in patients with bone metastases and correlate with each other, consistent with their relationship to skeletal involvement. While only OPN and bone-specific ALP are independent predictors of bone metastasis, OPN and PINP are the only independent negative predictors of survival in prostate cancer (35). In men with hormone-refractory prostate carcinoma there is a significant association between plasma OPN levels and indicators of tumor burden, including bone pain, weight loss, analgesic use, ECOG performance status, presence of metastases, and prior palliative therapy. ALP, total acid phosphatase, hemoglobin, lactate dehydrogenase (LDH), creatinine, and albumin, also are significantly correlated. Variables that are predictors of survival include the presence of carcinoma symptoms, weight loss, nausea and emesis, ECOG performance status, and prior palliative therapy, as well as levels of OPN, ALP, total acid phosphatase, hemoglobin, LDH, albumin, and aspartate aminotransferase (AAT) (36).

In prostate cancer, the expression of VEGF and HIF-1α; VEGF and OPN; OPN and HIF-1α correlate with each other. Increased expression of HIF-1α, VEGF, and OPN are each significantly associated with increased risk for biochemical treatment failure. On 2-year follow-up after radical prostatectomy, expression of VEGF and HIF-1α are significantly correlated with each other, but not with OPN expression (37).

There are robust correlations between *OPN* and *MMP9* gene expression and plasma levels in prostate cancer. OPN and MMP9 increase in patients with prostate cancer. MMP9 immunostaining is present in all tumor cells and in parts of the extracellular matrix. Very intense cyclooxygenase 2 (COX2) and OPN immunostaining are present in all cancer cells for all cases. Increased plasma levels of prostaglandin E_2, a metabolite of the COX2 enzyme, might be linked to the overexpression of COX2 in prostate cancer cells. Although prostaglandin E_2 is significantly increased in prostate cancer, there is only a marginal correlation between plasma levels of OPN, MMP9, and MMP2 with prostaglandin E (38).

The addition of markers to PSA may improve the assessment of prostate cancer. PSA, OPN and PINP are predictors of survival. OPN and bone-specific ALP are predictors of bone metastasis.

Renal cancer. In clear cell renal cell carcinoma, there is a strong association between the level of OPN expression and prognostic variables, including tumor size, grade, stage, and tumor growth fraction. (39) In metastatic renal cell carcinoma, OPN correlates positively with ICTP, bone-specific ALP, total ALP, and γ−glutamyltransferase (GGT) but negatively with hemoglobin and serum albumin. The levels of OPN, ICTP, and total ALP, as well as tumor stage, grade, and the presence of distant metastases, are significant univariate prognostic factors for death from renal cell carcinoma. OPN is superior to ICTP for the prediction of distant metastases (40). OPN, lost on transformation 1 (LOT1), heat-shock protein 90 (HSP90), casein kinase 1, thymosin b4, serum amyloid A, ceruloplasmin, SPARC, and CAP43 are overexpressed in the cancerous regions of renal cell carcinomas compared to the surrounding tissue, with overexpression of serum amyloid A, OPN, and HSP90 being marginal (41).

Oral cancer. In oral cancer, there is immunoreactivity for BSP within the cytoplasm and the perinuclear perimeters of proliferating tumor cells, with substantial intensity in well-differentiated tumor foci. Similarly, there is immunoreactivity for OPN in both the cytoplasm of malignant epithelial cells and in reactive immune cells infiltrating the connective tissue stroma. Dentin sialophosphoprotein (DSPP) immunoreactivity is confined to the cytoplasm and perinuclear perimeter of malignant epithelial cells alone. There are no differences between the expression of small integrin-binding ligand, *N*-linked glycoproteins (SIBLING) family gene products with histological differentiation, keratin profile, nuclear polymorphisms, number of mitoses, depth of invasion, pattern of invasion, regional lymph node spread, or stage of invasion among T1/T2 lesions. BSP and OPN levels are associated with median MMP2 and MMP3 levels. MMP3 and OPN are co-expressed in the cytoplasm of tumor cells. There are no significant correlations between OPN and MMP-9 levels (42). In a case of gingival spindle cell carcinoma with osteosarcomatous differentiation, the cells around bone-like calcified materials showed positive reaction for low-molecular weight cytokeratin and vimentin, whereas OPN was only positive in the bone matrix-like area. Cancer cells did not show positive reaction for OPN, BMP2 or BMP4 (43). In normal salivary glands, OSN is present in the striated ducts, while OPN is not expressed. Both markers are present in salivary pleomorphic adenomas. OSN is likely related to the production of type IV collagen by modified myoepithelial cells, whereas OPN is involved in the formation of myxoid and hyaline tissues (44).

Esophageal cancer. In esophageal squamous carcinoma, correlations between OPN and SCC or cytokeratin-19 fragment (CYFRA21-1) are weak. Individually, OPN, SCC, CYFRA21-1, or CEA are not independent indicators of survival. When combined, however, plasma OPN plus serum CYFRA21-1, and plasma OPN plus serum SCC antigen are independently prognostic (45). There is a correlation between RAS homolog gene family member C (RHO-C) and OPN expression in esophageal squamous carcinomas, and both are closely related to lymph node metastasis and survival time (46). OPN is overexpressed in all squamous cell carcinomas (by the tumor cells) and in 60% of adenocarcinomas (by infiltrating macrophages) of the esophagus. The expression of cathepsin L also varies with tumor histology, with overexpression in 60% of esophageal adenocarcinomas and 33% of squamous cell carcinomas (47).

Gastric cancer. Pepsin is one of the main proteolytic enzymes secreted by the gastric mucosa. It consists of a single polypeptide chain and arises from its precursor pepsinogen by removal of a 41 amino acid segment from the *N*-terminus. For gastric cancer screening, the specificity and positive and negative predictive values of OPN plus pepsinogen I are superior to those obtained by either marker alone (48). OPN expression is closely related to the invasion and metastases of gastric cancer. OPN may up-regulate the expression of the pro-invasive protease MMP9 by activating the NF-κB pathway. OPN, NF-κB p65 and MMP9 are absent from non-cancer gastric tissue, but are expressed in almost half of gastric cancer cases without lymph node metastasis. They increase to 75-80% in gastric cancer with lymph node metastases, and to 80-95% in gastric cancer with lymph node and distant metastases (49). OPN, COX2, and VEGF are overexpressed in gastric cancer compared to normal mucosa. The levels of all three markers are significantly correlated with each other and with tumor stage, as well as microvessel density, suggesting that OPN, COX2, and VEGF synergistically promote angiogenesis and metastasis (50). In gastric carcinoid tumor, OPN may be co-expressed with BMP, OSN, cytokeratin, chromogranin A, synaptophysin, neurofilaments, and neuron-specific enolase (51).

Colorectal cancer. Activation of the canonical wingless-type MMTV integration site family (WNT) pathway is reflected in an accumulation of cytoplasmic and nuclear β-catenin. OPN is a transcriptional target of the WNT pathway, and its expression is elevated in over 50% of colon tumors. OPN and β-catenin expression correlate in the majority of colon carcinomas, suggesting that they are related contributors to transformation. Whereas there is no elevated β-catenin expression without increase in OPN staining, 10% of carcinomas show elevated OPN expression in the absence of increased β-catenin. Several constituents of the WNT signaling cascade (including secreted frizzled-related protein-4, axin) as well as transcriptional targets of the WNT pathway (WISP1, cyclin D1), are differentially regulated in tumors as compared to normal colon. OPN is among the three transcripts with the highest up-regulation. The most strongly down-regulated target is carbonic anhydrase II, which is significantly decreased in all tumor stages (52). Genes that have a tendency to be overexpressed in colorectal carcinoma include OPN, interleukin 8 (IL-8), phosphatidylinositol glycan class F (PlGF), and angiogenin 2 (ANG2). Genes that are down-regulated include *VEGFD* (which is often undetectable in tumors, whilst always present in the surrounding mucosa), stromal cell derived factor 1 (*SDF1*), and CC chemokine ligand 5 (*CCL5*). Increase in OPN and decrease in CCL5 levels are predictors of lymph node invasion. Tyrosine kinase with Ig and EGF homology domains 2 (TIE2) and ANG2 also appear as discriminating predictors (53). The sequential overexpression of *OPN* and *OSN* mRNAs and proteins significantly correlates with the progression of the colorectal adenoma-dysplasia-carcinoma sequence (54).

Pancreatic cancer. The clinically used CA 19-9 is the best performing marker for pancreatic cancer. OPN, IGF2, SLPI, YKL40, and EGFR are comparably high-abundance markers (23). CA 19-9 and macrophage inhibitory cytokine 1 (MIC1) are indicators for the comparison of pancreatic cancer patients with normal healthy controls, with pancreatitis patients, and with non-cancer (benign disease and healthy) individuals. Unlike CA 19-9, MIC1 elevations in serum are independent of tumor stage in resectable pancreatic adenocarcinoma. Serum OPN is a less sensitive marker (55) .In pancreatic ductal adenocarcinoma associated with diffuse psammomatous calcification the tumor cells are positive for CK7, mucin-1 (MUC1), CEA, p53, and CA 19-9 (weak), and negative for MUC2 and chromogranin A. There is a strong positivity for OPN in the psammoma bodies, but not in the tumor cells or macrophages (56).

E-Cadherin and β-catenin are localized at the basolateral cell membrane of all exocrine cells in the pancreas. Their expression decreases in areas with inflammation. E-cadherin and β-catenin expression patterns are identical. In poorly differentiated ductal adenocarcinomas of the pancreas, there is moderate to strong OPN reactivity. Undifferentiated carcinomas without osteoclast-like giant cells and all undifferentiated carcinomas with osteoclast-like giant cells show cytoplasmic OPN expression. Tumor-associated macrophages are strongly positive for OPN in all cases. The extracellular tumor matrix expresses OPN only focally and weakly (57). In a rare case of osteoclast-like giant cell tumor associated with mucinous cystadenocarcinoma of the pancreas, osteoclast-like giant cells were positive for CD68, VEGF-C, and OPN (58).

Cancer of the ampulla of Vater may arise from epithelia of the common bile duct, the pancreatic duct, or the duodenal mucosa. The degree of OPN staining in these types of cancer does not correlate with tumor stage. By contrast, tumors expressing OSN show a trend toward more advanced stages, but OSN expression is not associated with tumor differentiation. Whereas stromal OSN expression is associated with greatly decreased survival, strong OPN staining does not relate to survival for ampullary cancer (59).

Liver cancer. α-Fetoprotein (AFP) blood levels are commonly used to detect and monitor liver cancer. The OPN overexpression in hepatocellular carcinoma (HCC) is closely associated with AFP elevation (60-62). One conflicting observation has been reported (63). OPN and AFP overexpression correlate with high-grade, high-stage tumors, early recurrence and lower survival. The aberrant expressions of AFP and OPN cooperatively contribute to tumor progression and poor prognosis and are useful for molecular staging of HCC (62). Tumor-related factors significantly associated with vascular invasion in HCC include the induction of OPN and serum AFP. Expression of OPN, serum AFP levels, grade, vascular invasion, tumor size, number of tumors, and Child-Pugh classification are significantly associated with long-term survival after hepatectomy (64). Among HCC patients, plasma OPN levels are significantly increased in correlation with the degree of liver function deterioration in terms of advanced Child-Pugh class. The area under the receiver operating characteristic (ROC) curve for plasma OPN is larger than that of AFP or prothrombin induced by vitamin K absence II (PIVKA II), suggesting that the diagnostic accuracy for HCC of plasma OPN is superior to that with AFP or PIVKA II. In HCC cases with non-diagnostic AFP levels, determining OPN levels could be especially useful. While the correlation between AFP and OPN is non-significant, the correlation between OPN and PIVKA II is significant (65). The frequent overexpression of tumor-associated trypsin inhibitor (TATI) and its correlation with HCC progression is shared by AFP and OPN, which also encode secreted proteins. AFP elevation occurs in 50%, and OPN overexpression in 55% of HCC cases. Portal vein invasion and early tumor recurrence are associated with overexpression of TATI, AFP, and OPN. These HCCs also display a lower 5-year survival rate (66).

Like OPN, the expression of adhesion molecules may affect tumor progression. OPN and intercellular adhesion molecule 1 (ICAM1) are detectable in the plasma of HCC patients. The levels significantly increase in patients with recurrence during a given follow-up time compared with those without recurrence. OPN and ICAM1 are predictors of overall survival and disease-free survival (67). The evaluation of preoperative plasma levels of OPN or ICAM1 may be helpful to predict the recurrence and prognosis of HCC patients in advance. The assessment of OPN level in combination with ICAM1 may stratify patients into groups with different potentials of HCC recurrence and different outcomes more accurately than OPN or ICAM1 individually (67).

Loss of E-cadherin, non-nuclear overexpression of β-catenin, and overexpression of OPN are significantly associated with tumors of higher histologic grade, but not with tumor size. The expression of E-cadherin mRNA but not *OPN* mRNA, in cirrhotic liver has a tendency to be higher than that in non-cirrhotic liver. Tumor size, grade, and overexpression of OPN are independent risk factors for vascular invasion. The expression of E-cadherin, but not OPN, has predictive value for recurrence within 12 months. Tumor size, grade, vascular invasion, number of tumors, expression of OPN, Child-Pugh classification, and expression of E-cadherin are significantly associated with long-term survival after hepatectomy (64)(68).

OPN and CD44 are expressed in a fraction of HCC cases. The overall survival and disease-free survival rates are significantly higher in the $OPN^{-}CD44^{-}$ group than in patients positive for one or both markers. The co-index CD44/OPN is an independent prognostic factor. When OPN and CD44 are taken into consideration together to predict tumor recurrence, the sensitivity and specificity are higher than for either parameter alone (63).

OPN overexpression in HCC is closely associated with *P53* mutation, tumor size, grade, stage, metastasis, early recurrence, and a lower survival rate. Mutation of *P53* and overexpression of OPN also correlate with stathmin overexpression. The great majority of HCCs harboring stathmin overexpression and concomitant *P53* mutation or OPN overexpression have vascular invasion (stage IIIA–IV). Furthermore, stathmin overexpression interacts with *P53* mutation or OPN overexpression to contribute to shortened survival (60, 69). OPN overexpression correlates with the down-regulation of ANXA10S, a short isoform of annexin, in HCC; both correlate with high-grade, high-stage tumors, early recurrence and reduced survival. The aberrant expressions of OPN and ANXA10S cooperatively contribute to tumor progression and poor prognosis and are useful for molecular staging (62). Several genes including *OPN*, glypican 3, spondin-2, *PEG10*, and *EDIL3* are overexpressed in HCC *versus* adjacent tissue, whereas ficolin 3 is the most consistently underexpressed gene. The top overexpressed genes in HCC compared to normal liver encode many proteins related to matrix, or matrix signaling, including *OPN*, *COL1A2*, collagen types III-V, *TSP*, *OSN*, SPARC-like 1 (Hevin), lumican, glypican 3, galectin 3, and versican (70).

In HCC, most studies have found a good correlation of AFP and OPN with each other as well as with tumor progression and poor prognosis. In HCC cases with non-diagnostic AFP levels, determining OPN levels could be useful. The co-evaluation of OPN with CD44, ICAM-1, or E-cadherin may be helpful to predict recurrence in HCC patients.

Lung cancer. In early stage non-small cell lung cancer, there is a significant inverse correlation between tumor-to-lung oxygen pressure and plasma OPN levels, suggesting that hypoxic tumors generally have higher OPN levels than those that are less hypoxic. Plasma OPN levels significantly correlate with CD44v6 immunohistochemistry staining and predict a higher risk of relapse (71). ALP, which consists of several isoforms including bone-specific ALP, is a maker for bone metastasis in several cancers. In advanced non-small cell lung cancer, there is a significant positive correlation between serum OPN and serum ALP (72). By contrast, there are no correlations between basal OPN plasma levels and either PAI1 or VEGF. Plasma levels of OPN, but not PAI1 or VEGF, are prognostic for patient outcomes. Forty percent of patients with low OPN levels, but only 25% of patients with high OPN respond to treatment. No such association exists for either PAI1 or VEGF. After one or two cycles of therapy, post-treatment plasma levels of OPN are not significantly reduced. In contrast, both PAI1 and VEGF levels drop. Changes in OPN, PAI1, or VEGF levels are not associated with subsequent progression-free survival or overall survival. Changes in OPN levels did not differ significantly between the control arm and the platinum-based chemotherapy arm of treatment (73). OPN concentrations in tumor and plasma are not significantly correlated. There is an inverse association between PAI1 plasma levels and tumor OPN (73). In curatively resected non-small cell lung cancer, there are no significant associations between OPN levels and smoking, development of metastases, tumor stage, or tumor grade. There is no correlation between the gene expression levels of OPN and SPARC (OSN). Thirty-five percent of patients have high expression only for OPN, 45% show a low expression status for OPN and SPARC, whereas high expression levels of both markers are present in 10% of patients. Co-expression is a significant unfavorable prognostic factor (74).

In pulmonary adenocarcinoma, OPN levels correlate with the levels of p-ERK and MMP9, but not with phosphorylated signal transduction and activation of transcription 3 (p-STAT-3), phosphorylated oncogene *AKT* (p-AKT), MMP1 or MMP2 (75). $P21^{RAS}$ and OPN are co-expressed in 30% of cases (76). The mean microvascular capillary density, reflected in the level of CD34, is significantly higher in OPN-positive than in OPN-

negative adenocarcinomas. The mean microvascular capillary density in VEGF- and OPN-positive adenocarcinomas is prominently high. The postoperative relapse rate in patients with VEGF-positive and OPN-positive stage I lung adenocarcinoma is extremely significantly higher than that in patients with VEGF-negative or OPN-negative stage I lung adenocarcinoma (77).

$P21^{RAS}$ and OPN are always co-expressed in lung squamous cell carcinoma, but rarely in small cell lung cancer (76). In squamous cell carcinomas of the lungs, OPN and VEGF are not associated with microvascular capillary density or prognosis (77).

The serum levels of OPN, mesothelin, and megakaryocyte potentiating factor are elevated in mesothelioma. OPN and megakaryocyte potentiating factor do not differentiate patients with mesothelioma from patients with other malignancies or with transudative pleural effusion. Combining these three markers does not improve sensitivity (78). There is a correlation between blood mesothelin and OPN values in malignant pleural mesothelioma, benign pleural lesions associated with asbestos exposure, and pleural metastasis by various carcinomas. However, the magnitude of the correlation between mesothelin and OPN is low among malignant pleural mesothelioma patients. There is also no correlation between pleural OPN and serum or plasma OPN. Although OPN is a high sensitivity marker with poor specificity while mesothelin has good specificity with a lower sensitivity, their combination does not improve patient classification over using mesothelin alone. In patients with pleural metastasis from various carcinomas, neither age, sex, primary tumor localization, serum or pleural mesothelin, nor serum or pleural OPN can predict survival. In malignant pleural mesothelioma patients, a significant relationship with survival exists for serum mesothelin as well as serum and plasma OPN (79).

OPN, CD44v6, ALP, and SPARC are markers for progression and risk of relapse. The mean microvascular capillary density can be assessed by the levels of CD34. The value of VEGF depends on the histologic type of lung cancer. PAI-1 may be reflective of initial treatment response.

Head and neck cancer. A large percentage of head and neck squamous cell carcinomas display strong tumor staining for OPN, dihydrofolate reductase (DHFR) and inhibitor of nuclear factor κ-B kinase subunit β (IKKβ). Only OPN tumor staining correlates with tumor partial oxygen pressure (pO_2). Positive IKKβ staining is significantly associated with a higher hypoxic fraction (80). In advanced head and neck cancer patients, there is a correlation between median tumor partial oxygen pressure and plasma OPN, but not between plasma OPN and HP2.5 (percentage of partial oxygen pressure readings below 2.5 mm Hg). HIF-1α correlates with carbonic anhydrase 9 (CAIX) and about 60% of the patients with elevated HIF-1α also have high plasma OPN. Among patients with high plasma OPN, 60% have severely hypoxic tumors. The association between hemoglobin concentration and plasma OPN is borderline significant (81). In patients after radiotherapy or combined chemoradiation, OPN tumor staining correlates with low hemoglobin, high HIF-1, and high serum VEGF levels. In stage IV patients, the median PO_2 is inversely correlated with the OPN expression. Yet, there is no correlation of OPN with HF5, HF2.5, or CAIX expression. (82) OPN expression alone has only a small impact on prognosis. However, the 5-year overall survival of patients whose tumors lack OPN, HIF-1 and CAIX is dramatically increased over patients in whose tumors any one of the markers is positive (82). At five years' post treatment, patients with high plasma OPN, HIF-1α or HP2.5 all have poor locoregional tumor control (81).

While OPN is absent from normal laryngeal tissue, its staining increases with the degree of dysplasia. OPN expression is significantly associated with diffuse CD44v6 staining. Although metaplastic areas are almost always negative for OPN and CD44v6, OPN and CD44v6 positivity are prognostic for the development of squamous cell carcinoma at the follow-up. OPN and CD44v6 expression levels in laryngeal dysplasia correlate negatively with disease-free survival and positively with progression and relapse (83). In head and neck squamous cell carcinomas there are significantly higher expression levels of OPN, MMP12, MMP1 and neurotensin (NTS) in cancer tissues compared to normal tissues. The high NTS expression group shows a reduced distant metastasis-free survival rate. There is no association of OPN, MMP12 and MMP1 expression with clinicopathological signatures or distant metastasis-free survival (84). In laryngeal squamous cell carcinoma, connective tissue growth factor (CTGF) tumor staining is significantly lower in cancer tissue than in surrounding tissue, but the converse is true for OPN staining. Whereas CTGF is significantly higher in the cases without metastasis of lymph node and clinical stage T1 than those in the ones with metastasis of lymph node and clinical stage T3, OPN has the inverse trend (85). In head and neck cancer patients, OPN levels do not correlate with SCC antigen levels. OPN levels are similar in SCC-positive and SCC-negative patients (86).

In head and neck cancer, OPN has been identified as a marker for partial oxygen pressure. Related indicators include HIF1α, CAIX, VEGF, and IKKβ. Some correlations exist among the levels of these biomolecules. CD44v6, NTS and CTGF could be combined with OPN as progression markers.

Glioblastoma. In glioblastoma, *OPN* mRNA is rather uniformly up-regulated, *CAIX* and *VEGF* exhibit up-regulation in about half of all cases. Common overexpression of CAIX and OPN occurs in about 15% of patients (87). In the glioblastoma microvasculature, VEGF-positive vessels are also positive for OPN and for integrin $\alpha_V\beta_3$ (88). Glioblastoma multiforme overexpresses genes that are all related to cell

adhesion and invasion. They include OPN, fibronectin, chitinase-3-like-1, keratoepithelin, fibromodulin, deleted in esophageal cancer 1 (DEC1), and possibly tissue inhibitor of metalloproteinase 3 (TIMP3). SPARC/OSN CWCV and Kazal-like domains proteoglycan (SPOCK) is a calcium-binding proteoglycan. An elevated ratio of OPN/SPOCK1 is strongly associated with the diagnosis of glioblastoma multiforme, with high specificity and sensitivity (89). According to serial analysis of gene expression (SAGE) of one untreated glioblastoma, gene transcripts of the macrophage-related genes OPN and MCP are overexpressed 12-fold and 8-fold, respectively, compared with normal brain tissue (90).

Skin cancer. Progression markers for melanoma in tumoral cells are OPN, N-cadherin, and OSN. Their expression is associated with an increased incidence of metastases (91). Expression of the α_v and β_3 integrin subunits and of OPN is stronger in melanomas than in benign nevi, whereas the expression of CD44 is weaker in melanomas than in nevi. OPN expression is associated with β_3-integrin staining and increased tumor cell proliferation by Ki67 (92).

Basal cell carcinoma with matrical differentiation is an extremely rare variant. Tumors express membraneous and cytoplasmic β-catenin. There is focal staining for OPN in basaloid cells. Surrounding macrophages stain for OPN and CD68 (93).

Thyroid cancer. Cystatin 6 (CST6) is overexpressed in 65% of papillary thyroid cancers, but is not expressed in follicular thyroid adenoma, most normal thyroid tissues, and follicular thyroid cancer. CXC chemokine ligand 14 (CXCL14) and short-chain dehydrogenase/reductase family member 3 (DHRS3) expression are elevated in 70% and 80% of papillary thyroid cancer cases, respectively, while being absent from normal adjacent thyroid tissues, most of follicular thyroid adenomas, and follicular thyroid carcinomas. Although brevican (BCAN) is present in 65% of papillary thyroid carcinomas, it is also expressed in several follicular thyroid adenomas and follicular thyroid cancer, suggesting that it is not specifically associated with tumorigenesis of papillary thyroid cancer. OPN, a transcriptional target gene induced by the oncogene RET/PTC, is expressed in 30% of papillary thyroid cancer cases. Interestingly, its levels are similar in 25% of follicular thyroid cancer cases. CST6 and CXCL14 tumor abundance is higher in metastatic than in non-metastatic papillary thyroid cancer. Although DHRS3 is expressed in both groups, its levels are elevated in non-metastatic papillary thyroid cancer. Although OPN has a higher expression in metastatic than non-metastatic papillary thyroid cancers, the difference is significant in papillary thyroid cancers with larger than 1 cm diameter (94). The vast majority of papillary thyroid carcinomas display intense OPN immunoreactivity. Classic papillary thyroid carcinomas are invariably positive also for CD44v6 (95). In papillary thyroid carcinoma, OPN expressing cells are present around psammoma bodies and constitute $CD68^+$ macrophages (96).

Sarcoma. In osteosarcoma, OPN and VEGF expression correlate with each other. Although osteosarcoma patients with high VEGF expression showed a trend towards shorter overall survival, OPN expression was not found to have a correlation with overall or disease-free survival (97).

Epithelioid osteosarcoma of the mandible shows positivity for OPN, OC, OSN, VS38c, and S100. Collagen I is focally positive for the extracellular matrix and malignant osteoid (98). Circulating tumor burden in osteosarcoma patients may be reflected in the mRNA levels of *OPN*, *OC*, and *OSN* in peripheral blood. Low collagen I mRNA levels are present in the peripheral blood of only 35% of healthy individuals, but significantly higher levels exist in over 90% of osteosarcoma patients (99).

In metastasizing osteosarcomas, TBS3 is elevated and serves as a predictor of worse overall survival, event-free survival and relapse-free survival at diagnosis. After chemotherapy, patients with tumors overexpressing TBS3 have worse relapse-free survival. High OSN expression is associated with most osteosarcomas. Its levels correlate with the worst event-free survival and relapse-free survival. Overexpression of OPN occurs in about 90% of osteosarcomas and is correlated with better overall survival, event-free survival and relapse-free survival at diagnosis (100).

In an osteosarcoma arising in the breast, the tumor cells expressed vimentin and CD10. About 10% of osteoblastic cells expressed OPN, showing a patchy distribution. Almost all neoplastic bone and malignant osteoids expressed OPN and VEGF. Osteoclastic cells expressed OPN, VEGF, ALP, CD68, α1-antitrypsin, and α1-antichymotrypsin (101). By contrast, epithelioid osteosarcoma of the mandible shows positivity for OPN but is negative for vimentin and cytokeratins (98).

The specific non-collagenous proteins of bone matrix OPN, BSP, and OC are present in the cartilage matrix of osteochondromas, but not in healthy cartilage. Throughout osteochondromas, most cells are proliferating cell nuclear antigen (PCNA)-positive, suggesting that osteochondroma chondrocytes present some characteristics of hypertrophic cells (collagen X expression) but they proliferate and fail to terminally differentiate (102).

Granular cell tumors are positive for OPN with immunostaining showing mostly diffusely distributed positive tumor cells, and only few or no positive macrophages or monocytes. In contrast, granular cells in granular cell epulides and other Schwannian cell tumors have no immunoreactivity for OPN in the tumor cells, but a few cells in the interstitium of granular cell epulis, neurilemmoma or malignant peripheral nerve sheath tumors are positive. Most of them are macrophages or monocytes that display positivity for CD68,

S100A8/S100A9 and negativity for S100 protein. All granular cell tumors have intense and diffuse immunoreactivity for S100, which is the same as neurilemmomas, amputation neuromas and about two thirds of malignant peripheral nerve sheath tumors. In addition, all granular cell tumors have intense and diffuse immunoreactions for CD68 in tumor cells, whereas there is scattered and weak staining in neurilemmomas and in one some malignant peripheral nerve sheath tumors. There is no reaction in amputation neuromas. The tumor cells of granular cell tumors show similar reactivity for OPN and KP1, and have a vesicular or granular appearance in their cytoplasm. The intracytoplasmic localization of both OPN and KP1 is almost identical (103).

The expression of *OPN*, hepatocyte growth factor receptor (*MET, HGFR*) and hepatocyte growth factor (*HGF*) mRNA is significantly increased in the tumor tissue of soft tissue sarcomas. There is a modest association between OPN and MET protein levels in these sarcomas. Whereas OPN is associated with higher tumor stage and grade, as well as decreased overall survival, MET is not (104). In primary sarcoma of the pulmonary artery, the majority of the spindle-shaped polymorphous tumor cells stain strongly positive for OPN, and most of these cells are double labeled with MIB1. An intracellular accumulation of OPN protein is present in $CD68^+$ macrophages. Healthy pulmonary arteries are negative for OPN protein or the standard form of CD44, but there is a focally positive membrane expression of CD44 splice variants in tumor-associated lymphocytes (105).

Myeloma. In myeloma, OPN, serum levels of ICTP, MIP-1α, and urine levels of deoxypyridinoline are significantly higher in patients with bone involvement than in patients without. There are significant correlations between OPN and LDH. Bone pain scores are correlated with OPN levels (106). The OPN serum concentration in myeloma patients correlates with the concentrations of creatinine, β_2-microglobulin, corrected calcium, hemoglobin, albumin and white blood cell count. OPN levels do not correlate with age, sex, number of osteolytic lesions, percentage of plasma cells in the bone marrow, immunoglobulin class, urine immunoglobulin and serum M-protein concentration, serum LDH, C-reactive protein (CRP), platelet counts or HGF (107).

Markers in Multiple Cancer Types

OPN and bone markers. OPN and OSN may be overexpressed in osteosarcoma (98), hepatocellular carcinoma (70), renal cell carcinoma (41), breast carcinoma (10) and melanoma (91). In normal salivary glands, OSN is present in the striated ducts, while OPN is not expressed. However, OPN and OSN are expressed in salivary pleomorphic adenomas (44). By contrast, in curatively resected non-small cell lung cancer there is no linear correlation between the gene expression levels of OPN and OSN (74).

OPN and OSN have been associated with tumor progression. The OPN and OSN mRNA levels in peripheral blood of osteosarcoma patients may reflect circulating tumor burden (99). While normal mammary epithelial cells have undetectable or weak immunoreactivity for OSN and OPN, their expression levels increase in the progression to infiltrating carcinomas (lobular and ductal types) (10). Their sequential overexpression also correlates with the progression of the colorectal adenoma-dysplasia-carcinoma sequence (54). The abundance of OPN and OSN is indicative for an increased incidence of melanoma metastases (91). However, in ampullary cancer, tumors expressing OSN, but not OPN, show a trend toward more advanced stage and nodal metastases (59).

OPN and OSN have limited value in prognostication. In curatively resected non-small cell lung cancer, co-expression of OPN and OSN is an unfavorable prognostic factor (74). The 5-year survival rates of OPN- and OSN-positive breast cancer patients are not significantly different (5). OSN levels in osteosarcoma patients correlate with the worst event-free survival and relapse-free survival, whereas OPN levels correlate with better overall survival, event-free survival and relapse-free survival at diagnosis (100). Whereas strong OPN staining does not relate to survival for ampullary cancer, stromal OSN expression is associated with greatly decreased survival (59).

The non-collagenous proteins OPN and OC are present in the cartilage matrix of osteochondromas, but not in healthy cartilage (102). Epithelioid osteosarcoma of the mandible shows positivity for OPN and OC (98). Circulating tumor burden in osteosarcoma patients may be reflected in the mRNA levels of OPN and OC in peripheral blood (99).

The gene expression levels of *BMP2* and *OPN* are relatively low in normal breast tissue. Whereas the *BMP2* mRNA level decreases in DCIS tissue, the *OPN* level increases. The average *BMP2* gene expression is approximately the same in bone metastatic tissue as in normal breast tissue (11).

OPN is expressed in a larger fraction of breast carcinomas than BSP. There are *BSP* and *OPN* transcripts in both invasive and *in situ* carcinoma components, which are not detectable in surrounding stromal cells or in peritumoral macrophages (12, 18). In breast cancer, the immunohistochemical staining pattern for BSP is frequently stronger in the cells lining the lumen of tumor glands but without the apical staining pattern for OPN. As is the case of OPN, infiltrating and poorly differentiated areas stain strongly for BSP. In primary tumors, the staining pattern of the two proteins does not differ between breast and prostate cancer (14). In bone metastases, OPN staining is significantly stronger in breast cancer metastases than in prostate cancer metastases, while BSP staining is significantly stronger in prostate cancer metastases than in breast cancer metastases (14). There is no correlation between OPN and BSP in breast cancer bone metastases (13).

BSP and OPN are present in the cartilage matrix of osteochondromas, but not in healthy cartilage (102).

While the gene expression levels of *OPN* and *OPG* are relatively low in normal breast tissue, and gene expression of *OPG* is unchanged between non-invasive and normal breast tissue, there have been conflicting results on the association between their levels in breast cancer. There was no difference in OPG and OPN serum values between patients with or without metastases. However, there were changes in OPN and OPG levels in patients with progressive disease. Patients with bone metastasis, but not patients without metastasis, showed a significant increase in both analytes during 12 weeks of anastrazole treatment (9, 11).

Plasma levels of OPN in breast cancer are positively correlated with serum ALP. In patients without bone metastases, a correlation between OPN and bone ALP arises only during anastrazole treatment. In patients with bone metastasis, there is a weak correlation between OPN and bone ALP, which is maintained during treatment (9, 15).

In men with hormone-refractory prostate carcinoma there is a significant association between the levels of plasma OPN and ALP as well as total acid phosphatase. OPN and bone-specific ALP are significantly higher in patients with bone metastases than in patients without. Predictors of survival include OPN, ALP and total acid phosphatase (36, 40).

OPN correlates positively with bone-specific ALP and total ALP in renal cell carcinoma. OPN and total ALP show an inverse correlation with survival time (40).

OPN and OSN levels, as well as OPN and ALP levels correlate in various types of cancer, where they are associated with tumor progression but not clearly with prognosis. The knowledge basis for OPN and OC or BMP2 is insufficient to draw conclusions. Published results for OPG are conflicting.

OPN and angiogenesis markers. OPN and VEGF expression correlate with each other in gastric cancer (50), prostate cancer (37) and in osteosarcomas (97, 101). OPN and VEGF are overexpressed in gastric cancer. Their levels correlate with stage, as well as microvessel density (50). In glioblastoma micro-vasculature, VEGF-positive vessels are also positive for OPN and for Integrin $\alpha_V\beta_3$ (88). In advanced head-and-neck cancer treated with radiotherapy or combined chemoradiation, there is a significant correlation of positive OPN staining with high serum VEGF level (82). The mean microvascular capillary density (measured by levels of CD34) in OPN-positive adenocarcinomas of the lungs is significantly higher than that in OPN-negative adenocarcinomas, whereas there is no significant difference between OPN-positive and OPN-negative squamous cell carcinomas. The mean micro-vascular capillary density in VEGF-positive and OPN-positive adenocarcinomas is prominently high, whereas that in VEGF-positive and OPN-positive squamous cell carcinomas is not (77).

By contrast with the preceding examples, in advanced non-small cell lung cancer patients, there are no correlations between basal OPN plasma levels and VEGF (73). In colorectal carcinoma, genes with a tendency to be overexpressed in the tumor include *OPN* and *ANG-2*, whereas *VEGFD* is down-regulated (53).

OPN and VEGF have been assessed with regard to disease outcome. In prostate cancer, increased expression of VEGF and OPN are each significantly associated with decreased freedom from biochemical treatment failure (37). The postoperative relapse rate in patients with VEGF-positive and OPN-positive stage I lung adenocarcinoma is substantially higher than that in patients with VEGF-negative or OPN-negative stage I lung adenocarcinoma, unlike patients with stage I lung squamous cell carcinoma. Patients with VEGF-positive and OPN-positive adenocarcinoma have significantly worse prognosis than those that are negative for one or both markers. But there are no significant differences according to OPN or VEGF expression in the survival curves among patients with squamous cell carcinoma (77). In advanced non-small cell lung cancer patients, plasma levels of OPN, but not VEGF, are prognostic for patient outcomes. Of patients with low OPN levels, 40% respond to treatment, whereas only 25% of patients with high OPN respond to treatment. No such association exists for VEGF. After one or two cycles of therapy, post-treatment plasma levels of VEGF, but not OPN, are significantly reduced. Changes in OPN or VEGF levels are not associated with subsequent progression-free survival or overall survival (73). Although osteosarcoma patients with high VEGF expression show a trend towards shorter overall survival, OPN expression has no correlation with overall or disease-free survival (97).

Hypoxic tumors generally seem to have higher OPN levels than those that are less hypoxic. In head and neck cancer patients as well as in early stage non-small cell lung cancer, there is an inverse correlation between pO_2 and plasma or tumor OPN levels (71, 80-82). In advanced head-and-neck cancer treated with radiotherapy or combined chemoradiation, a significant correlation exists of positive OPN staining, high HIF-1 expression and low hemoglobin level. A low median pO_2 tends to occur more frequently in patients with positive OPN staining (82). Whereas *OPN* mRNA is rather uniformly up-regulated in glioblastoma, compared to normal brain and to low-grade astrocytoma, CAIX, which catalyzes the hydration of carbon dioxide, exhibits a strong up-regulation in about half of all tumors. Both proteins are up-regulated in about 15% of patients (87). HIF-1α correlates with CAIX, and about 60% of head and neck cancer patients with >50% HIF-1α also have high plasma OPN. The association between hemoglobin concentration and plasma OPN is borderline significant (81). In prostate cancer, expression of OPN and HIF-1α are significantly correlated with each other (37).

OPN expression alone has only a small impact on head and neck cancer prognosis. However, the 5-year overall survival of patients whose tumors show neither expression of OPN nor HIF-1 nor CAIX is significantly higher than if any one of the markers is positive (82). At five years' post treatment, head and

neck cancer patients with high plasma OPN, HIF-1α or low pO_2 have poorer locoregional tumor control (81). Increased expression of HIF-1α or OPN in prostate cancer is significantly associated with increased biochemical treatment failure. On 2-year follow-up after radical prostatectomy, expression of HIF-1α was not correlated with OPN expression (37).

OPN and VEGF correlate with each other and with microvascularization in some cancer types. However, this marker combination may not be useful for prognostication. OPN is often up-regulated in tumor hypoxia and tends to correlate with other markers of hypoxia.

OPN and cytoskeleton/motility markers. As a mediator of invasiveness, OPN may interact with components of the cytoskeleton and regulators of motility. One of its receptors is variant CD44. 85% of classic papillary thyroid carcinomas and 100% of the tall cell variant papillary thyroid carcinoma tumors display intense OPN immunoreactivity. Classic papillary thyroid carcinomas are also positive for CD44v6 (95). In early stage non-small cell lung cancer, plasma OPN levels significantly correlate with CD44v6 immunohistochemistry staining and predict a higher risk of relapse (71). While OPN is absent from normal laryngeal tissue, its staining increases with the degree of dysplasia. Of all mild dysplasias, 20% are highly positive for OPN, which is significantly associated with a diffuse CD44v6 staining. $OPN^+CD44v6^+$ areas of metaplasia are prognostic for the development of squamous cell carcinoma. OPN staining and full thickness CD44v6 positivity in laryngeal dysplasia correlate negatively with disease-free survival and positively with risk for relapse (83). In hepatocellular carcinoma, OPN and CD44 expression do not correlate with each other. Expression of OPN or CD44 compromises the overall survival and disease-free survival rates compared to OPN^-CD44^- patients. The co-index CD44/OPN is an independent prognostic factor for survival. For predicting tumor recurrence, the sensitivity and specificity of OPN and CD44 combined is higher than that for either parameter alone (63). The expression of OPN is stronger in melanomas than in benign nevi, whereas the expression of CD44 is weaker in melanomas than in nevi (92).

The ERM complex, composed of ezrin, radixin, and moesin, is associated with the homing molecule and OPN receptor CD44. In endometrial carcinoma, there is a significant correlation of strong ezrin expression with primary tumor stage and overall survival. However, there is no correlation between ezrin expression and OPN expression. The 5-year survival rate is reduced for patients with strong ezrin-expressing tumors (31).

There is a positive correlation between RHO-C and OPN expressions in esophageal squamous carcinoma patients. Both markers are closely related to lymph node metastasis and survival time (46). Glioblastoma multiforme *versus* pilocytic astrocytoma overexpresses genes that are all related to cell adhesion and invasion. They include OPN, fibronectin, chitinase-3-like-1, keratoepithelin and fibromodulin (89). Overexpression of OPN correlates with stathmin overexpression in hepatocellular carcinoma, and is associated with higher vascular invasion, and lower 5-year survival rate. Stathmin overexpression is associated with frequent vascular invasion, regardless of the presence or absence of OPN overexpression. The great majority of hepatocellular carcinomas harboring stathmin overexpression and concomitant OPN overexpression have vascular invasion (stage IIIA–IV) (108). In osteosarcoma arising in the breast, the tumor cells express vimentin and CD10. About 10% of osteoblastic cells expressed OPN, showing a patchy distribution. Almost all neoplastic bone and malignant osteoids expressed OPN (101).

OPN and adhesion molecules. In hepatocellular carcinoma, overexpression of OPN, loss of E-cadherin and non-nuclear overexpression of β-catenin are associated with tumor grade but not with tumor size. Whereas OPN and E-cadherin are predictors of long-term survival after hepatectomy, the level of E-cadherin expression is reflective of the risk for recurrence within 12 months (64, 68). E-cadherin and β-catenin are localized at the basolateral cell membrane of all exocrine cells in the pancreas. Their expression decreases in areas with inflammation. In poorly differentiated ductal adenocarcinomas, there is moderate to strong OPN reactivity. The E-cadherin and β-catenin expression patterns are identical to OPN (57). N-Cadherin and OPN in tumoral cells are markers for melanoma. Their expression is associated with an increased incidence of metastases (91). Basal cell carcinoma with matrical differentiation is an extremely rare variant. Tumors express membraneous and cytoplasmic β-catenin. There is focal staining for OPN in basaloid cells and surrounding macrophages (93).

OPN and ICAM1 are present in the plasma of hepatocellular carcinoma patients. The levels are significantly elevated in patients who have recurrence compared with those without recurrence. OPN and ICAM1 are predictors of reduced overall survival and disease-free survival. The preoperative plasma levels of OPN or ICAM1 may be helpful to predict the prognosis of hepatocellular carcinoma patients. The assessment of OPN in combination with ICAM1 could stratify patients into groups with distinct outcome prospects (67). Both OPN and CEACAM1 are expressed in endometrial cancer, and both interact with Integrin β_3. CEACAM1 is co-expressed with OPN in normal endometrium and in endometrial neoplasia, implying the possibility that the two molecules may form a functional complex (30).

Expression of the α_v and β_3 integrin subunits and of OPN is stronger in melanomas than in benign nevi. OPN expression is associated with β_3-integrin staining and increased tumor cell proliferation, and it is significantly increased in metastatic lesions compared with their corresponding primary nodular melanomas (92).

All CA125-deficient ovarian carcinomas express OPN and CLDN3 (an integral membrane protein important in tight junctions) at some level (24).

OPN and extracellular matrix molecules. Circulating tumor burden in osteosarcoma patients may be reflected in the mRNA levels of *OPN* and type I collagen in peripheral blood. Type I collagen mRNA is present in only 35% of healthy individuals, but is significantly higher in over 90% of osteosarcoma patients (99). *OPN* mRNA exists in 60% of non-invasive DCIS that express pro-collagen mRNA, and the levels are generally comparable. At baseline in breast cancer, there is a weak correlation between OPN, ICTP and cross-linked PINP, which is maintained during anastrazole treatment (9, 16). Concentrations of ICTP and PINP do not differ among healthy men, benign prostate hyperplasia patients, or prostate cancer patients with or without lymph node affliction, while OPN shows a higher concentration in benign prostate hyperplasia patients than in healthy men. About 80% of metastatic prostate cancer patients have increased OPN values compared to 70% of patients with increased values of ICTP and 65% increased PINP, and there are correlations among all three markers. OPN, ICTP and PINP are effective for the detection of bone metastases, with OPN and PINP being significant predictors of bone metastasis while ICTP has less impact. These markers are potential prognostic factors for survival in prostate cancer patients (40). While OPN and ICTP levels do not change from healthy individuals to node-negative renal cancer, their levels in the renal cell carcinomas with regional lymph node and distant bone and non-bone metastases are significantly elevated. While OPN correlates with ICTP, it has superior diagnostic accuracy over ICTP for the prediction of distant metastases. The levels of OPN or ICTP, as well as tumor stage, grade, and the presence of distant metastases, are significant univariate prognostic factors for death from renal cell carcinoma (35). In myeloma, serum levels of OPN and ICTP are significantly elevated in patients with bone involvement (106). The list of the top overexpressed genes in hepatocellular carcinoma comprises many proteins related to matrix, or matrix signaling, including collagen 1A2, collagen type IV, collagen type III, collagen type V and OPN (70).

The most overexpressed genes in hepatocellular carcinoma comprise many proteins related to matrix or matrix signaling, including TSP and OPN (70). Breast tumor cells synthesize OPN and TSP1, THBS1. Normal and non-malignant breast epithelia and stromal elements express lower levels of OPN, while TSP1 is essentially absent. However, TSP1 levels are significantly higher in all primary tumors and in all metastases over normal breast epithelia. In line with the OPN patterns of expression, there is no significant difference in TSP1 levels between primary tumors with or without metastases, nor between primary carcinomas and their associated metastases (17). In osteosarcomas, TSB3 (THBS3) is expressed at significantly high levels in patients with metastasis at diagnosis, which is a predictor of worse overall survival, event-free survival and relapse-free survival at diagnosis. Over-expression of OPN occurs in about 90% of osteosarcomas and may correlate with better overall survival, event-free survival and relapse-free survival at diagnosis (100).

Collagen can be processed into diverse forms. The peptides ICTP and PINP may correlate with OPN as well as with tumor dissemination in multiple types of cancer. Whether additional information can be gained from measuring all three markers in blood or tumor tissue needs to be further investigated. TSP1 may correlate with OPN in some cancer types.

OPN and molecules of the fibrinolytic system. Some OPN functions are mediated by uPA (109). In advanced non-small cell lung cancer patients, there are no correlations between basal OPN plasma levels and PAI1. However, there is an inverse association between PAI1 plasma levels and tumor OPN. Plasma levels of OPN, but not PAI1, are prognostic for patient outcomes, with low OPN levels being associated with a higher fraction of responders to therapy. In contrast, after 1-2 cycles of therapy post-treatment plasma levels of PAI1, but not OPN, show significant reductions. Changes in OPN or PAI1 levels are not associated with subsequent progression-free survival or overall survival (73). There is no correlation between the development of bone metastases and the expression of the uPA system in breast cancer. *uPAR* mRNA is higher and *PAI1* mRNA lower in the bone metastases compared to their matching primary tumors. OPN mRNA expression varies considerably between primary and secondary tumors (16). In osteosarcoma arising in the breast, about 10% of osteoblastic cells express OPN, showing a patchy distribution. Almost all neoplastic bone and malignant osteoids express OPN. Osteoclastic cells express OPN, α1-antitrypsin, and α1-antichymotrypsin (101).

OPN and gene products of the fibrinolytic cascade generally do not correlate with each other. In sertain types of cancer, the fibrinolytic molecules may add prognostic information that cannot be obtained from OPN measurements.

OPN and SCC. For the detection of cervical cancer, there is a correlation between OPN and SCC antigen levels. However, the sensitivity and specificity of plasma OPN are better than those of SCC antigen. There is a significant increase in sensitivity over SCC alone when combining it with OPN (32). In esophageal squamous cell carcinoma, correlations among OPN, SCC, and CYFRA21-1 are weak. Patients with high plasma OPN plus high serum SCC have the worst prognosis and the patients with low OPN and low SCC have the best prognosis. Patients with high plasma OPN or high serum CYFRA21-1 have a worse prognosis than patients with low OPN and low CYFRA21-1. Individually, OPN, SCC, CYFRA21-1, or CEA are not independent

indicators of survival (45). In head and neck cancer patients, OPN levels do not correlate with SCC antigen levels. OPN levels are similar between SCC-positive and SCC-negative patients (86).

OPN and regulators of calcium homeostasis. S100 proteins are small, acidic proteins that transduce Ca^{2+} signals *via* interactions with intracellular target proteins. While immunocytochemical staining for S100A4, present in 70% of minimally invasive breast carcinomas, correlates with higher vessel density, there is no such association for OPN staining, which is detectable in 50% of cases. Each marker is associated with poorer survival times. The relative risk for patients with OPN-positive tumors with or without S100A4 is greater than that for patients with S100A4-positive tumors with or without OPN. Patients with both S100A4 and OPN in the primary tumors show a reduction in survival time over those with either one alone. Lymph node status, tumor size, histologic grade, and vessel density are associated with a reduction in patient survival time over that with S100A4 or with OPN alone. There is a significant association of primary breast cancer staining for S100P with the prognostic markers S100A4 and OPN. The corresponding association in carcinoma in the axillary lymph nodes is a weak. Independent predictors of outcome are tumor staining for S100A4 followed by that for OPN, involved lymph nodes, and S100P (5, 8).

Epithelioid osteosarcoma of the mandible shows positivity for OPN and S100 (98). Granular cell tumors are positive for OPN, with immunostaining showing mostly diffusely distributed positive tumor cells and few or no positive macrophages or monocytes. In contrast, granular cells in granular cell epulides and other Schwannian cell tumors have no immunoreactivity for OPN in the tumor cells, but a few positive macrophages or monocytes that also stain for CD68 and S100A8/S100A9. All granular cell tumors have intense and diffuse immunoreactivity for S100, which is the same as neurilemmomas, amputation neuromas and about two thirds of malignant peripheral nerve sheath tumors (103).

In renal cell carcinoma, the levels of OPN and corrected serum calcium as well as the tumor stage and grade are prognostic factors for reduced survival time (35).

Two isoforms of the calcitonin receptor result from alternative splicing of the primary mRNA transcript and differ by the inclusion or exclusion of a 16-amino-acid insert within the first putative intracellular loop. The insert-negative calcitonin receptor mRNA is expressed by all breast carcinomas. In contrast, only 40% of tumors express the insert-containing calcitonin form. *OPN* mRNA is present in all breast carcinomas. The expression of calcitonin and OPN is limited to the tumor cells and absent from the surrounding stroma (18).

OPN and the NF-κB pathway. OPN and NF-κB P65 are not expressed in non-cancer gastric tissues. Their expression rates increase in transformation and progression to metastatic gastric cancer, with correlations between the two markers. OPN may up-regulate the expression of the pro-metastatic molecule MMP9 *via* the NF-κB pathway (49). In head and neck squamous cell carcinoma, there is strong staining in a large percentage of tumors for OPN and in a very small percentage for IKKβ. While OPN tumor staining correlates to median tumor pO_2, IKKβ staining is associated with a higher hypoxic fraction (80). The gene expression levels of *OPN* and *RANKL* are relatively low in normal breast tissue. There are no significant correlations between these markers and histology or tumor size of the primary breast cancer. The average gene expression levels of *RANKL* are no different in non-invasive and normal breast tissue (11).

OPN and secreted proteases. In primary oral squamous cell carcinoma, there are correlations between OPN and MMP2, as well as MMP3, levels. However, there are no correlations between OPN and MMP9 (42). Conversely, in pulmonary adenocarcinoma, there are correlations between OPN and MMP9 but not between OPN and MMP1 or MMP2 (75). OPN and MMP9 are absent from non-cancer gastric tissue samples, but their expression rates increase with gastric cancer progression. OPN expression is closely related to invasion and metastases. It may up-regulate the expression of MMP9 by activating the NF-κB pathway (49). In prostate cancer patients, OPN and MMP9 levels are significantly higher compared with those from benign prostate hyperplasia patients and healthy controls, and significantly higher in benign prostate hyperplasia patients compared with healthy controls. There is a robust correlation between *OPN* and *MMP9* gene expression and plasma levels (38). In head and neck squamous cell carcinomas, there are higher expression levels of OPN, MMP1 and MMP12 in cancer tissues compared to normal tissues. However, there is no association between OPN, MMP1, or MMP12 with clinicopathological signatures or distant metastasis-free survival (84). The blood derived markers OPN and MMP7 are diagnostic for serous ovarian carcinoma (22).

OPN is overexpressed in 100% of esophageal squamous cell carcinomas and in 60% of esophageal adenocarcinomas relative to normal esophageal mucosa. By comparison, cathepsin L is overexpressed in 60% of primary esophageal adenocarcinomas and 33% of squamous cell cancers (47).

Pepsin is one of the main proteolytic enzymes secreted by the gastric mucosa. It consists of a single polypeptide chain and arises from its precursor, cepsinogen, by removal of a 41-amino acid segment from the *N*-terminus. For gastric cancer screening, the specificity and predictive values of OPN plus pepsinogen I are superior to those obtained by pepsinogen I alone. With OPN alone, only good specificity can be achieved (48).

Table II. *Tumor-specific marker combinations*.

Marker	Cancer
CA125	Ovarian
PSA	Prostate
CA 19-9	Pancreatic
AFP	Liver
SPOCK1	Glioblastoma
SCC	Squamous cell carcinoma

OPN may be up-regulated together with various MMPs in cancer. Their combined detection could enhance the assessment of tumor progression, and may in some cases facilitate tumor detection. For gastric cancer screening, OPN plus pepsinogen I has superior specificity and predictive values compared to either marker alone.

Conclusion

Despite a substantial volume of publications on the subject of OPN plus other markers in cancer diagnosis (800 papers on OPN and cancer evaluated, 228 included in the meta-analyses, 115 identified here to report the combination of OPN with other biomarkers), the literature is mostly inconclusive. Even though some trends can be identified, such as the correlation of OPN with PSA in prostate cancer, OPN and CA125 in ovarian cancer, OPN and AFP in liver cancer, most population sizes are small and it is not uncommon that multiple reports come to conflicting conclusions. In some cases, such as the correlation between OPN and ER in breast cancer, the published results are mutually inconsistent.

OPN is a cancer progression marker with high sensitivity but limited specificity, as it is elevated in about 30 different types of cancer (1, 2). Although blood and tumor levels do not always correlate, OPN from both sources has promise as a diagnostic and prognostic indicator that reflects tumor progression and is associated with reduced survival. Its combination with biomarkers that are specific for individual cancer types (Table II) could add value in cancer detection.

Many of the studies reviewed here have investigated correlations between OPN and other biomarkers in cancer. Indicators that correlate with OPN could be used to strengthen the diagnostic reliability of OPN but they add little additional information. In contrast, readouts for other characteristics of transformation, such as markers for hypoxia, angiogenesis, or bone affliction, may be more valuable in marker panels with OPN. Yet, for many candidate combinations, insufficient published evidence is available. No algorithms have been developed that facilitate clinical decisions based on marker combinations that involve OPN. Tightly controlled, large-scale studies will be required for the full potential of OPN in diagnostic multiplex panels for cancer to come to fruition.

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