The Biology Behind


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The protein now known as OPN2 was first identified and characterized in two contexts: (a) in bone as a phosphorylated sialoglycoprotein (1); and (b) in cultures of transformed cells as transformation-related phosphoprotein (2). The rat cDNA encoding the bone protein was cloned from an osteosarcoma cell line and named OPN because of its ability to form a bridge between bone cells and bone matrix (3). Mouse OPN was cloned as 2ar, a cDNA clone identified in a differential screen as corresponding to a mRNA that was strongly induced in mouse JB6 epidermal cells by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (4). Thus from the time it was first discovered, both as a protein and as a cDNA, there was the implication that OPN might be important in cancer biology. It is interesting that at about the same time a number of laboratories cloned OPN in a number of different contexts, for example as Eta-1 (early T-lymphocyte activation gene 1; Ref. 5) and as 2B7, a rat smooth muscle cell mRNA elevated in contexts, for example as Eta-1 (early T-lymphocyte activation gene 1; Ref. 5) and as 2B7, a rat smooth muscle cell mRNA elevated in transformation-related phosphoprotein (2). The rat cDNA, there was the implication that OPN might be important in cancer biology. It is interesting that at about the same time a number of laboratories cloned OPN in a number of different contexts, for example as Eta-1 (early T-lymphocyte activation gene 1; Ref. 5) and as 2B7, a rat smooth muscle cell mRNA elevated in the carotid artery after balloon angioplasty (6). The human cDNA was cloned and characterized by Young et al. (7). Why was this gene identified at about the same time in so many different contexts? The price of the secreted protein, OPN mRNA levels must be substantially induced in a short time to produce sufficient protein to impact significantly on the extracellular environment.

OPN was subsequently discovered to be a Ras-responsive gene; it was ras responsive in that its mRNA levels were substantially increased in Ras-transformed cells or after Ras activation (8–10). Fueger et al. (11) have reviewed the involvement of OPN in malignant processes, with a focus on human breast cancer. Le et al. (12) now report in this issue of Clinical Cancer Research that OPN is a prognostic plasma marker for HNSCCs. This is an exciting development because it provides a new diagnostic for solid tumors that have been notoriously difficult to relate to a plasma marker. HNSCC is unfortunately a rather common malignancy that has been difficult to treat successfully. Using a powerful machine learning algorithm (Linear Discriminant Analysis), Le et al. (12) searched a microarray expression database for a gene that could identify cell lines expressing high levels of the VHL tumor suppressor gene product. VHL disease develops from germ-line mutations and is characterized by highly vascularized tumors that can develop in various parts of the body (13).

The VHL protein is of interest because of its ability to modulate hypoxia-induced gene expression (14). HIF-1α protein is proteolyzed by an oxygen-dependent ubiquitination mechanism, limiting its ability to stimulate expression of hypoxia-induced genes such as vascular endothelial growth factor and transforming growth factor α (15). Under normoxic conditions, specific prolines in HIF-1α are hydroxylated by prolyl hydroxylase using molecular oxygen. The VHL protein binds to the hydroxyproline, stimulating polyubiquitination of HIF-1α and subsequent proteasome-mediated proteolysis. Under hypoxic conditions, proline hydroxylation is reduced, allowing the HIF-1α protein sufficient time to accumulate and stimulate transcription of target genes. Because the VHL protein reduces HIF-1 expression, Le et al. (12) hypothesized that analysis of VHL-dependent gene expression might provide a clue to the function of HIF-1α in tumor cells.

The NCI-60 cancer cell line microarray expression data set, consisting of 60 cancer cell lines, was analyzed by Le et al. (12) for genes whose expression was predictive of VHL expression status in the data set. This analysis uncovered OPN as a gene whose expression inversely correlated with VHL expression, suggesting that OPN might be linked to tumor hypoxia. The mean plasma OPN level in VHL-negative patients was 447 ng/ml (range, 261–843 ng/ml), whereas control patients had a mean value of 318 ng/ml (range, 233–461 ng/ml). Le et al. (12) then investigated the correlation between plasma OPN levels and tumor pO2 in patients with HNSCC. Plasma OPN concentration was increased maximally in the serum of patients with hypoxic tumors; those patients with the highest OPN levels were most subject to tumor recurrence and a negative outcome.

Senger et al. (16) reported that 10 of 13 patients with disseminated carcinomas contained, relative to controls, elevated levels of OPN in their plasma as judged by Western blotting. Some years later, using a sensitive ELISA assay, Singhal et al. (17) reported that elevated OPN levels in patients with metastatic breast cancer were associated with an increased number of tumor sites and decreased patient survival. Other reports have noted correlations of OPN with malignancies in gastric cancer and human glioma (18, 19). Several recent studies have exploited microarray analysis to correlate OPN mRNA levels with cancer progression. Building on a cDNA microarray study, Kim et al. (20) validated OPN as a marker for ovarian cancer, confirming elevated mRNA levels in ovarian tumors and an association between plasma levels of OPN protein and ovarian cancer. Agrawal et al. (21) used a sample pooling strategy and oligonucleotide array analysis to identify genes whose expression correlated with colon cancer progression. Among the genes studied, OPN was most consistently differentially expressed in conjunction with tumor progression, suggesting its utility as a clinically useful marker.

Why OPN? How does hypoxia enhance its expression, and what is its role in tumor progression? It was noted as early as 1994 (22) that hypoxia (and subsequent reoxygenation) can increase

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2 The abbreviations used are: OPN, osteopontin; HNSCC, head and neck squamous cell carcinoma; VHL, Von-Hippel Lindau; HIF-1α, hypoxia-induced factor-1α.
OPN expression in proximal tubule epithelial cells. More recently, Sodhi et al. (23) have reported that exposure of cultured rat aortic vascular smooth muscle cells to hypoxia results in a biphasic enhancement of OPN mRNA levels, with peaks at about 2 and 24 h. The basis for increased OPN expression under hypoxic conditions is not known. With regard to its role in tumor progression, a number of possibilities have been suggested (24). OPN is both a cell attachment molecule and a cell signaling molecule, able to engage a number of receptors including several integrins and CD44 variants. Genes whose expression has been reported to be affected include inducible nitric oxide synthase, nuclear factor κB, vascular endothelial growth factor, urokinase-type plasminogen activator, and Met, the receptor for hepatocyte growth factor (11, 24). OPN, which is strongly up-regulated in many inflammatory processes, can support cell survival, stimulate cell migration, increase expression of other genes involved in invasive events, notably matrix metalloproteinases, and promote tumor angiogenesis (25–27). In short, the evidence is overwhelming that OPN contributes to tumor progression and aggressiveness in many circumstances. This is the significance of the finding by Le et al. (12) that the elevated OPN levels accompanying hypoxic HNSCC are indicative of a poor prognosis.

References


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