

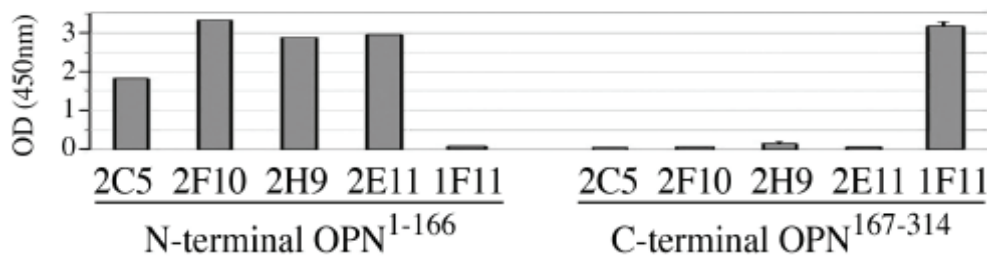
Monoclonal Antibodies to Osteopontin

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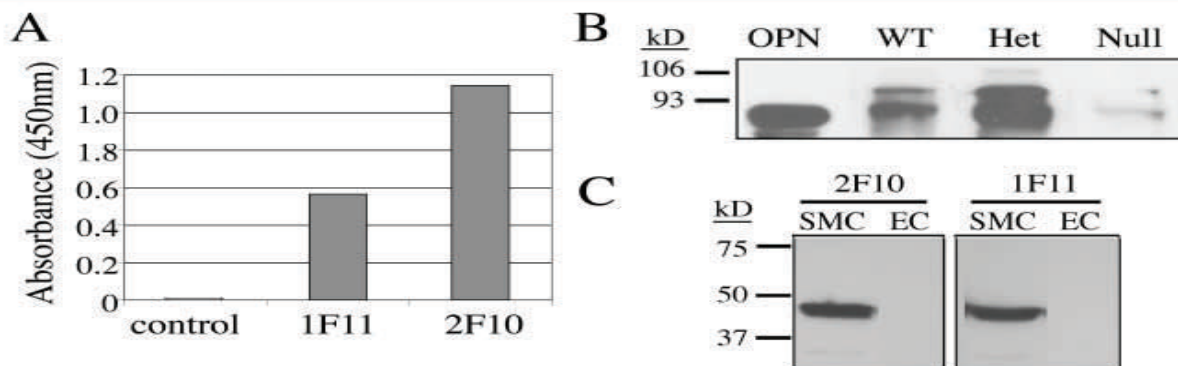
Osteopontin (OPN) is a well-characterized secreted protein found in the circulation and is emerging as a potential biomarker for many cancers. Maine Biotechnology Services, Inc. (MBS), in collaboration with Maine Medical Center Research Institute (MMCRI) and the University of Southern Maine (USM), developed five anti-human OPN monoclonal antibodies for use in research studying cancer and other OPN-related pathologies. MBS has manufactured, characterized and commercialized these five clones and made them available to the R&D market. Four of the five clones recognize the N terminal of OPN and one recognizes the C terminal of OPN. All Five clones recognize full length OPN.

ELISA Data



An ELISA was performed using the human N-terminal (aa-1-166) or C-terminal (aa167-314) human recombinant fragments. 2C5, 2H9, 2F10, and 2E11 recognize OPN epitopes on the N-terminal fragment, while 1F11 recognizes an epitope on the C-terminal fragment. All 5 clones recognize fl-OPN. (1)

Recognition of Native OPN

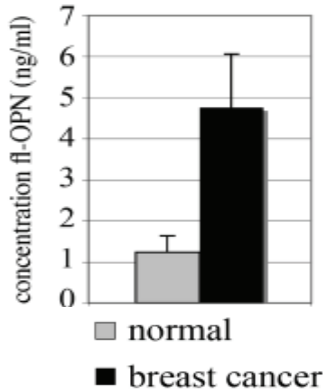


A) Human milk OPN was used in ELISA, and 1F11 and 2F10 binding compared to an irrelevant antibody (MAb 1E3, control). **B)** Whole kidney lysates were collected from wild type (WT) mice, OPN heterozygous mice (Het), and OPN null mutant mice (Null). Lysates were loaded equally and analyzed by SDS-PAGE followed by immunoblotting using 1F11. Recombinant OPN was used as a control. **C)** Human aortic smooth muscle cells (SMC) were transduced with activated Notch1 receptor (2) to increase OPN expression, and compared to human umbilical vein endothelial cells (EC). Both 2F10 and 1F11 recognize OPN in SMC.

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OPN Link to Breast Cancer



In a recent publication from MMCRI researchers, MAbs 2F10 and 1F11 were used to quantify plasma fl-OPN levels in fifty healthy volunteers (normal) versus forty patients with metastatic breast cancer (Stage IV). A significant increase was found in the cancer patient population. This indicates that more research on the potential for OPN as a biomarker is necessary.

While full-length OPN is biologically active in regulating cancer cell behavior, recent data suggests that a post-translational proteolytic cleavage event by matrix metalloproteinases (3) is an activating step in protein function. Our collaborators at MMCRI hypothesize that the biologically relevant cancer biomarker is an N-terminal proteolytic fragment of OPN, which has enhanced adhesive and migratory activity on tumor cells. MBS and MMCRI have already begun the development process for clones specific to this N-terminal proteolytic fragment and will be offering that product soon.

All assay results and data interpretation in this paper came from:

Alicia Plumer, Hongyi Duan, Sripriya Subramaniam, F Lee Lucas, Susan Miesfeldt, Ah-Kau Ng, Lucy Liaw Development of fragment specific osteopontin antibodies and ELISA for quantification in human metastatic breast cancer
BMC Cancer 2008, 8:38 (31 January 2008)

(1) Christensen B, Nielsen MS, Haselmann KF, Petersen TE, Sorensen ES: Posttranslationally modified residues of native human osteopontin are located in clusters: identification of 36 phosphorylation and five O-glycosylation sites and their biological implications. *Biochem J* 2005, 390(Pt 1):285-292.

(2) Havrda MC, Johnson MJ, O'Neill CF, Liaw L: A novel mechanism of transcriptional repression of p27kip1 through Notch/HRT2 signaling in vascular smooth muscle cells. *Thromb Haemost* 2006, 96(3):361-370

(3) Gao YA, Agnihotri R, Vary CP, Liaw L: Expression and characterization of recombinant osteopontin peptides representing matrix metalloproteinase proteolytic fragments. *Matrix Biol* 2004, 23(7):457-466

Antibodies For Research Use Only

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