ONCOblot Reports

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ENOX2: A Potential Target for Early Cancer Intervention

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Experts agree that early detection is a key component for any strategy to effectively reduce cancer-related morbidity and mortality. By detecting cancer early, the opportunity arises to treat the disease at what is considered the most curative stage, prior to metastatic spread. Unfortunately, other than surgical intervention, there is very little in the current Standard of Care appropriate for early intervention. Out of necessity, current Standards of Care are largely focused on coping with late stage cancer after presentation of clinical symptoms and frequently, after the cancer has spread beyond the primary site. Therefore, there is a great need for the development of safe and effective biological, pharmaceutical, nutraceutical and/or nutritional interventions that lack dose-limiting toxicities and are applicable to the treatment of early stage cancer.

The target molecule detected by the ONCOblot test is the ENOX2 cancer marker. Expression of the ENOX2 protein is restricted to cancer cells and is absent from normal cells and tissues. ENOX2 is currently the only protein marker thus far documented to be consistently produced by all of the most common forms of human cancer. ENOX2 is a cell surface protein that localizes to the outer plasma membrane of cancer cells. However, partially due to the fact that ENOX2 lacks a full transmembrane domain, ENOX2 is shed from cancer cells into the circulation. Interestingly, tissue-specific isoforms of ENOX2 are produced by cancers of different tissues of origin. Consequently, the combination of the number of unique ENOX2 isoforms, and molecular weight and isoelectric point of each ENOX2 isoform present within blood serum are indicative of the cancer tissue of origin. Therefore, the ENOX2 serum marker has utility for identification of the tissue of cancer origin, as demonstrated by the analysis of sera samples from clinically-confirmed cancer patients (1).

When associated with the outer leaflet of a cell plasma membrane, enzymes of the ENOX protein family perform functions that are critical to the growth phase of cell proliferation. For cancer cells, the constitutively active ENOX2 enzyme functionally replaces ENOX1, which is present on all cells and is highly regulated by both growth factors and hormones. The unregulated activity of ENOX2 strongly contributes to the characteristic unregulated growth and invasive phenotype that is common to most, if not all, forms of human cancer. The universal response of cancer cells in tissue culture when the enzymatic activities of ENOX2 are blocked is to undergo programmed cell death (apoptosis). As such, the ENOX2 proteins may serve as ideal molecular targets for early cancer intervention.

There is considerable evidence to support the above concept. For example, when ENOX2

was exogenously produced within MCF-10A (breast) or CHO (kidney) non-cancer cell lines, these cells gained an invasive phenotype (2). In contrast, when ENOX2 expression was silenced within a HeLa (cervical) cancer cell line, the invasive potential of these cancer cells was significantly reduced. These results suggest that ENOX2 expression is both necessary and sufficient for unregulated growth and invasive ability of immortalized cell lines.

In order to proliferate, cells must enlarge. If cells do not reach a critical size, they are unable to pass a checkpoint in G_1 that monitors cell size. Unregulated cancer cells, unlike most normal cells, undergo programmed cell death (apoptosis) if unable to grow and divide within 48 to 72 h (3, 4). Consistently, inhibitors of ENOX2 have been shown to selectively induce apoptosis in cancer cells through blocking the growth-related activities of this cell surface enzyme. Two such ENOX2 inhibitors are Epigallocatechin gallate (EGCg) found in green tea and capsaicin, a pungent molecule produced by chili peppers. EGCg has been shown to inhibit the proliferation of both BT-20 (breast cancer) and HeLa (cervical cancer) cells in culture, and the enzymatic activity of ENOX2 with an IC_{50} in the nanomolar range (4). Likewise, capsaicin inhibits the proliferation of BT-20 (breast cancer) cells, and inhibits ENOX2 activity with an IC_{50} in the nanomolar range as well (5). In contrast, the growth of the non-cancer MCF-10A (breast) cell line was not inhibited by either of these small molecules. Thus, ENOX2 may provide an effective pan-cancer intervention target that can be inhibited by compounds lacking any significant dose-limiting toxicities.

Importantly, not all very early cancers may develop into a life threatening disease. Therefore, aggressive treatment of early cancers remains controversial. While increasing the frequency at which cancers are detected early is predicted to lead to an increased rate of curative treatment with respect to the detection of the same disease at a later stage of disease progression, some very early cancers may not require treatment at all. Presently there is no way of knowing which early neoplasms will progress to life threatening cancers and which will not. With caution as a guiding principal, ENOX2 offers opportunities to develop new and effective intervention strategies designed to eliminate early malignancies. The overall concept is that of Curative Prevention®, where early detection and early intervention would be combined to prevent both invasive spread of the cancer as well as further development of the cancer into a life threatening condition.

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