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Early Detection: An Opportunity for Cancer Prevention Through Early Intervention

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1. Introduction

Cancer is the second leading disease cause of death in the United States. A group of more than 100 different and distinctive diseases, cancer may involve any tissue of the body. Estimates are that there were over 1.5 million cases in 2010 in the United States alone. Only a small fraction (less than 20%) of cancers are diagnosed at a localized stage where curative therapy is effective. Most cancers are diagnosed only after the primary tumor has already metastasized so that chemotherapy is required for treatment. Hence, early detection is a favored opportunity to reduce cancer mortality. By detecting cancer in its very earliest stages when perhaps only a small number of cells are present, it is possible that early intervention will be effective in preventing further development of the incipient cancer thereby resulting in what might be viewed as curative prevention.

Despite advances in early detection of major forms of human cancer (prostate, breast, lung, colon, leukemia, lymphoma), more often than not, cancers have developed to a sufficiently late stage at the time of detection to preclude most opportunities for curative therapy (Altekruse et al., 2010). The problem is exacerbated for pancreatic cancer where clinical symptoms invariably are delayed until the disease state is well advanced beyond metastatic spread. A need for early detection remains as one of the most important challenges at the forefront of cancer research, treatment and prevention.

2. Approach

2.1 Early detection

Ecto-Nicotinamide Adenine Dinucleotide Oxidase Disulfide-Thiol Exchanger 2 (ENOX2) (GenBank accession no. AF207881; Chueh et al., 2002) also known as Tumor-Associated Nicotinamide Adenine Dinucleotide Oxidase (tNOX) is ideally suited as a target for early diagnosis of cancer as well as for early preventive intervention (Fig. 1). The proteins are expressed on the cell surface of malignancies and detectable in the serum of patients with cancer (Cho et al., 2002). ENOX2 proteins are terminal hydroquinone oxidases of plasma membrane electron transport. From the standpoint of early intervention, they are important in the growth and enlargement of tumor cells (Morré and Morré, 2003a; Tang et al., 2007, 2008). Our approach using ENOX2, as a target for both early detection and for early interventions, is based on these properties (Cho et al., 2002; Morré and Morré, 2003a;

reviewed by Davies and Bozzo, 2006). While ENOX2 presence provides a non-invasive approach to cancer detection, without methodology to identify cancer site-specific ENOX2 forms, it offered no indication as to cancer type or location.

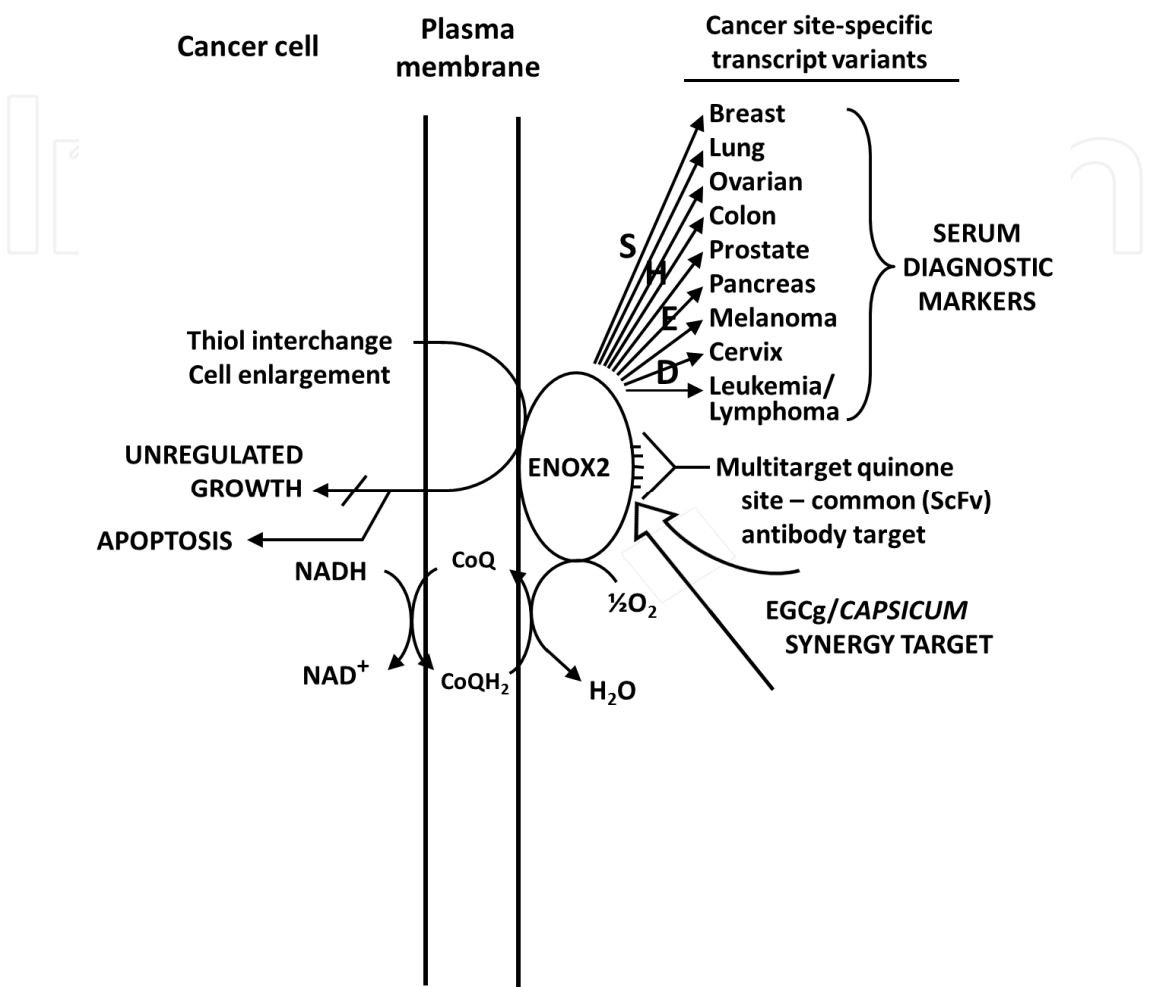


Fig. 1. Schematic representation of the utility of the ENOX2 family of cancer-specific, cell surface proteins for early diagnosis and early intervention of cancer. Cancer site-specific transcript variants of ENOX2 are shed into the serum to permit early detection and diagnosis. The ENOX2 proteins of origin at the cell surface act as terminal oxidases of plasma membrane electron transport functions essential to the unregulated growth of cancer. When the ENOX2 proteins are inhibited, as for example through EGCg/*Capsicum* synergies, the unregulated growth ceases and the cancer cells undergo programmed cell death (apoptosis).

The opportunity to simultaneously determine both cancer presence and cancer site emerged as a result of 2-dimensional gel electrophoretic separations where western blots with a pan ENOX2 recombinant single chain variable region (ScFv) antibody carrying an S tag (Fig. 2) was employed for detection (Hostetler et al., 2009; Hostetler and Kim, 2011). The antibody cross reacted with all known ENOX2 forms from hematological and solid tumors of human origin but, of itself, did not differentiate among different kinds of cancers. Analyses using this antibody, when combined with two-dimensional gel electrophoretic separation, revealed specific ENOX2 species possibly as transcript variants, each with a characteristic

molecular weight and isoelectric point indicative of a particular form of cancer (Hostetler et al., 2009; Table 1).

ENOX transcript variants of specific molecular weights and isoelectric points are produced uniquely by patients with cancer. The proteins are shed into the circulation and have the potential to serve as definitive, non-invasive and sensitive serum markers for early detection of both primary and recurrent cancer in at risk populations with a low incidence of false positives, as they are molecular signature molecules produced specifically by cancer cells and absent from non-cancer cells.

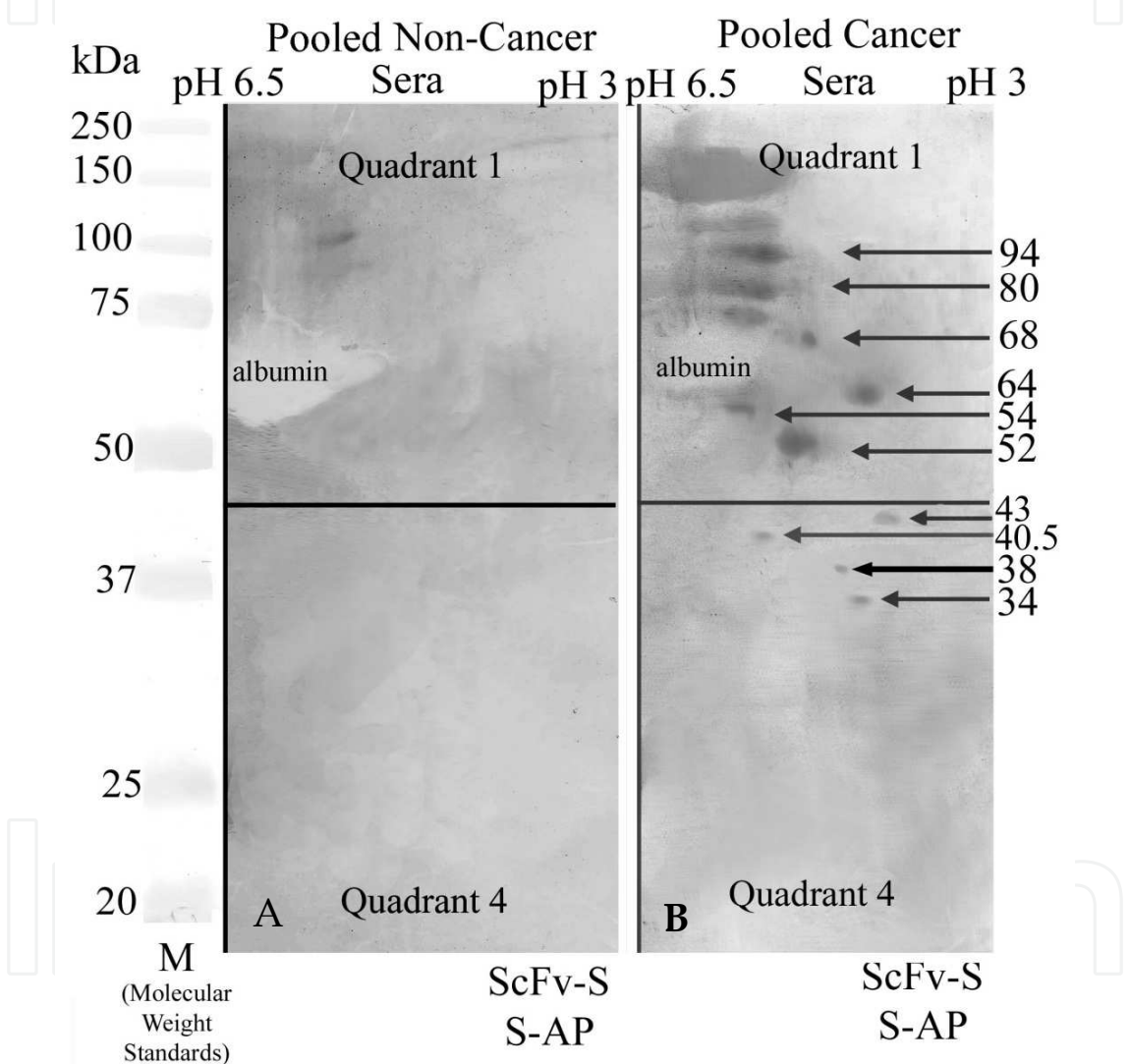


Fig. 2. 2-Dimensional gel/western blot of ENOX2 transcript variants comparing pooled non-cancer (A) and pooled cancer representing major carcinomas plus leukemias and lymphomas (B) patient sera. The approximate location of unreactive (at background) albumin is labeled for comparison. ENOX2 reactive proteins are restricted to quadrants I and IV. Detection uses recombinant scFv-S (S-tag peptide: His-Glu-Ala-Ala-Lys-Phe--Gln-Arg-Glu-His) antibody with alkaline phosphatase linked antiS protein. The approximately 10 ENOX2 transcript variants of the pooled cancer sera are absent from non-cancer (A) and are cancer site-specific as indicated in Fig. 3. From Hostetler et al. (2009).

Cancer	Sera analyzed	Molecular weight	Isoelectric point, pH
Cervical	18	94 kDa	5.4
Ovarian	41	80 and 40.5 kDa	4.2 and 4.1
Prostate	70	75 kDa	6.3
Breast/Uterine	55	64 to 68 kDa	4.5
Non-small cell lung	83	54 kDa	5.1
Small cell lung	22	52 kDa	4.3
Pancreatic	24	50 and 52 kDa	4.3 and 3.9
Colon	55	52 and 34 kDa	4.3 and 3.9
Lymphoma, Leukemia	16	45 kDa	3.9
Melanoma	12	38 kDa	5.1

Table 1. Sera from patients with different cancers exhibit distinct patterns of ENOX2 isoforms with characteristic molecular weights and isoelectric points (pH). Updated from Hostetler et al. (2009).

2.2 Early intervention

As the 2-D-western blot protocol detects cancer early, well in advance of clinical symptoms, the opportunity to combine early detection with early intervention as a potentially curative prevention strategy for cancer by eliminating the disease in its very earliest stages is unique. The approach to early intervention is based on previous work in cell culture models showing that ENOX2 proteins are required to support the unregulated growth that typifies cancer cells. If the growth function of ENOX2 is blocked for 48 to 72 h, the cancer cells cannot enlarge following division, cannot pass the checkpoint in G₁ that monitors cell size and eventually undergo programmed cell death (apoptosis) (Morré and Morré, 2003b; De Luca et al., 2010) as diagrammed in Figure 1. Among the early intervention strategies under investigation are several targeted to ENOX2, production of ENOX2-directed vaccines being one promising example. Recombinant ENOX2 peptides that exhibit cancer specificity are employed as antigens. Other forms of ENOX2-directed therapeutic interventions under study include use of dietary modulators (Morré et al., 2009b). Most advanced are studies with herbal mixtures of green tea and powders of ground chili peppers (*Capsicum* species) from efficacious pepper sources (e.g. guajillo or ancho) with levels of capsaicin, the pungent principle of chili peppers, sufficiently low so as to not cause discomfort.

3. Results

3.1 Early detection

Analytical 2-D gel electrophoresis and immunoblotting of ENOX proteins from a mixed population of cancer patients (cervical, breast, ovarian, lung and colon carcinomas, leukemias and lymphomas) revealed multiple species of acidic proteins of molecular weights between 34 and 100 kDa in quadrants I and IV (Fig 2B), none of which were present in sera of non-cancer patients (Fig. 2A) (Hostetler et al., 2009). Separation in the first dimension was by isoelectric focusing over the pH range of 3 to 10 and separation in the second dimension was by 10 percent SDS-PAGE. Isoelectric points of the ENOX2 transcript variants were in the range of 3.9 to 6.3. The principal reactive proteins other than the ENOX2 forms were a 53 kDa isoelectric point pH 4.1, mostly phosphorylated α 1-antitrypsin inhibitor

(α 2-HS-glycoprotein; fetuin A) (Labeled “R” in Fig. 5) which served as a convenient loading control and isoelectric point reference and a 79-85 kDa, isoelectric point pH 6.8 serotransferrin which served as a second point of reference for loading and as an isoelectric point reference (Table 2). The two cross reactive reference proteins are present in a majority of sera and plasma of both cancer and non-cancer subjects. Albumin and other serum proteins do not react. On some blots, the recombinant scFv was weakly cross-reactive with heavy (ca. 52 kDa) and light (ca. 25 kDa) immunoglobulin chains.

ENOX2	EEMTETK400 <u>E</u> TEESA406LVS
Alpha-1-antitrypsin inhibitor	GTDCVAK211 <u>E</u> ATEAA216KCN
Serrotansferrin	CLDGTRK589PVEEYA595NCH

Table 2. Protein sequence similarity between ENOX2 and the two reference proteins α 1- anti-trypsin inhibitor and serrotransferrin reactive with the pan ENOX2 scFv recombinant antibody. Regions of similarity are restricted to a 7 amino acid sequence (underlined) adjacent in ENOX2 to the E394EMTE398 quinone inhibitor-binding site.

Sera from individual patients with various forms of cancer were analyzed by 2-D gel electrophoresis and immunoblotting to assign each of the ENOX2 isoforms of Fig. 2 to a cancer of a particular tissue of origin (Table 1). Sera of breast cancer patients contained only the 64 to 68 kDa ENOX2 (Fig. 3D; Fig. 5 arrow) and the α 1-antitrypsin inhibitor reference protein (Fig. 5). Sera from cervical cancer patients contained the 94 kDa ENOX2 transcript variant (Fig. 3A). Sera from ovarian cancer patients contained ENOX1 transcript variants of 80 kDa and 40.5b kDa (Fig. 3B). Sera from patients with prostate cancer contained one or more 75 kDa ENOX2 transcript variants resulting in small variations in isoelectric points (Fig. 3C). Sera from patients with non-small cell lung carcinoma contained a 52 kDa ENOX2 transcript variant while sera from non-small cell lung carcinoma patients contained a 52 kDa ENOX2 transcript variant (Fig 3E;F; Fig 4). Simultaneous presence of ENOX2 transcript variants of both 50 and 52 kDa characterized sera of pancreatic cancer patients (Fig. 3G) whereas sera of colon cancer patients contained ENOX2 transcript variants of 52 kDa and 43 kDa (Fig. 3H). Fig. 3I from sera of a patient with non-Hodgkin’s lymphoma illustrates the 45 kDa ENOX2 transcript variant of low isoelectric point characteristic of leukemias and lymphomas. Sera of patients with malignant melanoma contained an ENOX2 transcript variant of 38 kDa (Fig. 3J).

Particularly relevant are observations where the 64 to 68 kDa ENOX2 transcript variant (pH 4.5) of sera correlated with disease presence in both late (Stage IV) (Fig. 5A) and early (Stage I) (Fig. 5E) disease and in Stage IV recurrence (Fig. 5C) but was absent from sera of non-cancer (normal) volunteers (Fig. 5B) or in survivors free of disease for one to five years (Fig. 5D). Additionally, the 64 to 68 kDa breast cancer-specific transcript variant does not apply to a subset of breast cancer patients but appears to be widely present. Analyses of sera of more than 55 patients with active disease including 20 Stage I and Stage II breast cancer patients all tested positive.

Unlike most published cancer markers, cancer-specific ENOX2 variants are not simply present as elevated levels of a serum constituent present in lesser amounts in the absence of cancer. The cancer-specific ENOX2 transcript variants result from cancer-specific expression of alternatively spliced mRNAs (Tang et al., 2007; 2008). Neither the splice variant mRNAs nor the ENOX2 isoform proteins are present in detectable levels in non-cancer cells or in sera of subjects without cancer (Table 1).

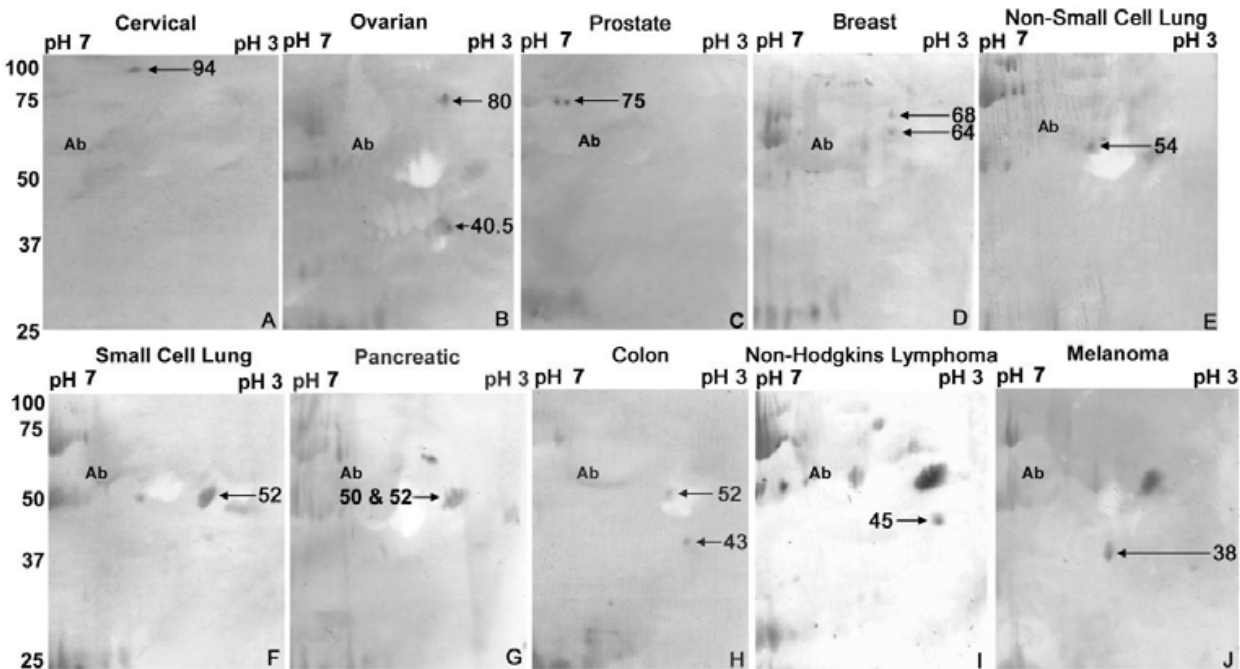


Fig. 3. Western blots of 2-D gel electrophoresis/western blots of sera from cancer patients analyzed individually. Cancer sites are presented in the order of decreasing molecular weight of the major transcript variant present. A. Cervical cancer. B. Ovarian cancer C. Prostate cancer. D. Breast cancer. E. Non-small cell lung cancer. F. Small cell lung cancer. G. Pancreatic cancer. H. Colon cancer. I. Non-Hodgkins lymphoma. J. Melanoma. The approximate location of unreactive (at background) albumin (Ab) is labeled for comparison. Approximately 180 non-cancer patient sera were analyzed in parallel without evidence of proteins indicative of specific transcript variants. From Hostetler et al. (2009).

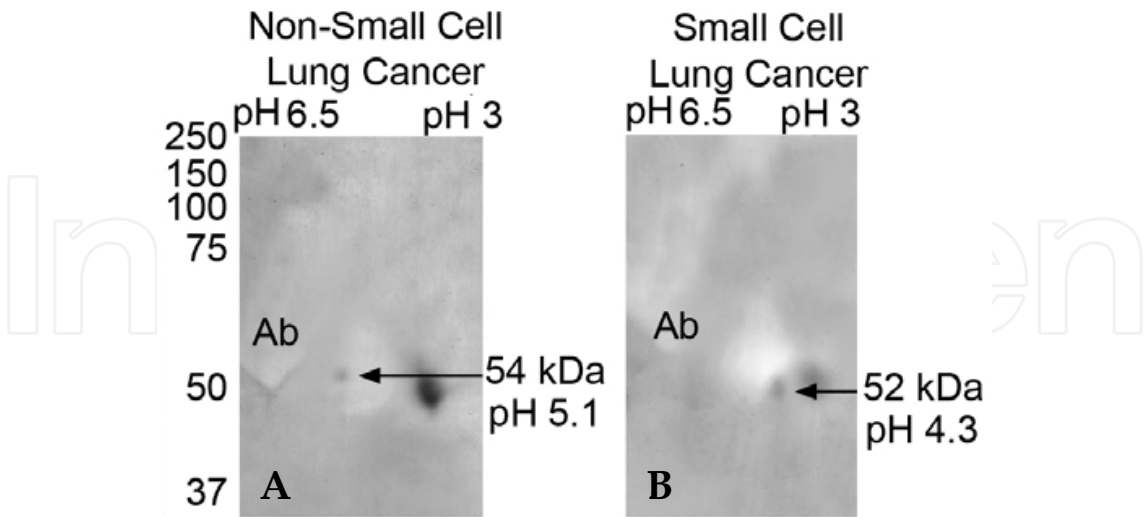


Fig. 4. Analytical gel electrophoresis and immunoblot of patient sera. A Sera from a patient with non-small cell lung cancer contains a 54 kDa ENOX2 transcript variant, pH 5.1 (arrow). B. Sera from a patient with small cell lung cancer contains the 52 kDa, isoelectric point pH 4.3 is transcript variant (arrow). The reference spots to the right, M_r 52 kDa and isoelectric point pH 4.1 is α 1-antitrypsin. Albumin and other serum proteins are unreactive.

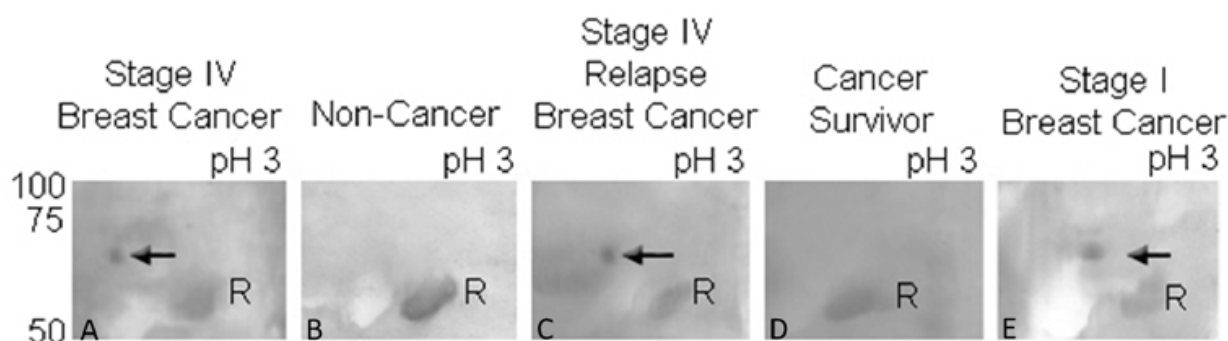


Fig. 5. 2-D gel electrophoretic separations and detection of ENOX2 transcript variants specific for breast cancer by western blotting of patient sera. Arrow = 66 to 68 kDa breast cancer specific transcript variant. R = 52 kDa, isoelectric point pH 4.1 α 1-antitrypsin inhibitor reference spot.

Findings from a separate study with small cell and non-small cell lung cancer suggest that the 2-D-western blot test detects cancer presence as early as 5 to 7 years in advance of the appearance of clinical symptoms. This supposition is based mainly on our analysis of two special cancer panels of sera obtained through the Early Detection Research Network (EDRN) of the National Cancer Institute. One panel consisted of about 20 known lung cancer patient sera and 35 control patient sera. Using the 2-D-western blot protocol to identify specific ENOX2 isoforms, we successfully identified all 20 of the known lung cancer patient sera. However, unexpectedly, a high incidence of ENOX2 presence was encountered in sera from the "control" group which were obtained from a community screening study. From additional information obtained through the EDRN, 16 of the 17 positive control subject samples where our findings specifically indicated lung cancer (the lung cancer ENOX2 markers were found) were smokers with smoking histories in the range of 15 to 88 pack-years. However, the anticipated incidence of undetected lung cancers in such a population would be in the order of 10% or less rather than nearly 50%. Since the aberrant ENOX2 transcript variants associated with lung cancer are single molecular species produced only by lung cancer, the possibility was raised that lung cancer was being detected much earlier than was currently possible by other methods. The indications might be as early as 5 to 7 years before clinical symptoms, based on the estimated 20 year development time for lung cancer expression between carcinogen exposure and a clinically evident cancer (Petro et al., 2000) as diagrammed in Figure 6.

Similar results were obtained with a panel of female subjects at risk for breast and ovarian cancer. An analysis of a panel of 127 sera in a Biomarker Reference Set for Cancers in Women also provided through the Early Detection Research Network of the National Cancer Institute support our indications that the 2-D gel-western blot system is able to detect cancer presence 5 to 7 years in advance of clinical symptoms. The panel consisted of samples pooled from 441 women in 12 different gynecologic and breast disease categories plus 115 sera from age-matched control women. Of the 127 sera samples in the panel, 29 tested positive for breast cancer and another 16 tested positive for ovarian cancer. Since the aberrant transcript variants are single molecular species produced by specific cancers such as lung, breast or ovarian, the findings suggest that cancer was being detected in the control population much earlier than is currently possible by other methods. As estimated for lung

cancer, the indications might be as much as 5 to 7 years before clinical symptoms based on the development time estimated for breast as well as lung cancer expression between a cancer causing event and clinically evident disease (Weinberg, 2007).

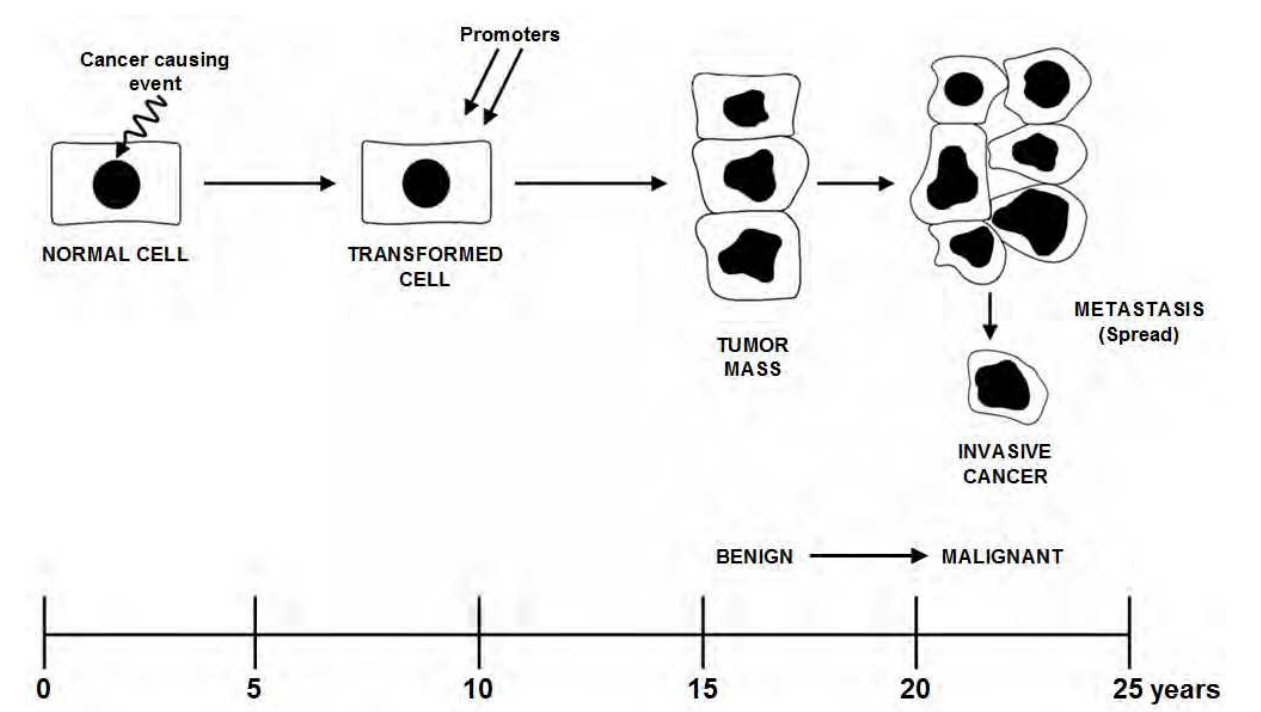


Fig. 6. Interpretive diagram to illustrate the various stages of cancer progression (estimated to require as long as 20 y) beginning with a cancer-causing event (initiation) through development of a clinically defined malignancy.

4. Discussion

4.1 Significance

Cells in tissues and organs are continuously subjected to oxidative stress and free radicals as well as other potential cancer initiating events on a daily basis (Kryston et al., 2011). Cells normally withstand these attacks but some result in cancer causing events to initiate the rather long (est. 20 y) development phase prior to clinical symptoms (Fig. 6). Our hypothesis is that ENOX proteins being critical to the unregulated growth of cancer will be shed into sera well in advance of clinical symptoms as the rationale for the proposed early detection strategy. The essential role of ENOX2 in unregulated cancer growth provides the basis for early intervention.

Early detection coupled with early intervention as diagrammed in Figure 1 raises the possibility of an important paradigm shift in cancer management toward early diagnosis and treatment options vastly different from those currently employed to deal primarily with advanced cancer. Consequently, the treatment of cancer might evolve from a primarily acute to a more chronic setting with monitoring and less invasive treatments. Reducing surgery, radiation and chemotherapy, as well as shortened hospital stays based on less invasive and less costly interventions afforded by very early detection would be expected to have a significant impact on reducing health care costs world wide.

Applications of the diagnostic methodology to post surgery patients is expected to help determine which patients still harbor residual disease following surgery and will require chemotherapy and which patients are free of disease where chemotherapy could be delayed or averted. The expectation is that the 2-D gel-western blot protocol will indicate that chemotherapy might be avoided or delayed in many patients (cancer survivors) where no evidence of disease is indicated. Additionally, the assay might be employed in patients with no clinical evidence of disease to monitor for recurrence.

4.2 ENOX2 cloning and tissue distribution

ENOX2 was expression cloned (Chueh et al., 2002) (Genbank Accession No. AF207881) using a monoclonal antibody that recognizes only a common ENOX2 epitope near the cancer drug-binding site (Cho et al., 2002) and from which the pan ENOX2 scFv recombinant antibody was derived (Kim, 2011). This binding site contains a conserved 5 amino acid (EMTEE) motif (Table 2). Based on biochemical (drug inhibition of activity) and immunological evidence, this EEMTE drug binding motif in Exon 5 appears to be common to all ENOX2 forms and absent from the amino acid sequence of the constitutive ENOX1 proteins characteristic of both cancer and non-cancer cells.

The presence of ENOX2 proteins in sera of cancer patients represents an origin due to shedding from the patient's cancer (Wilkinson et al., 1996). The presence of the ENOX2 proteins has been demonstrated in a number of human tumor tissues and xenografts (mammary carcinoma, prostate cancer, neuroblastoma, colon carcinoma, and melanoma). However, serum analysis indicates a much broader association with cancer. ENOX2 proteins are ectoproteins reversibly bound at the outer leaflet of the plasma membrane (Morré, 1995). As is characteristic of other examples of ectoproteins (sialyl and galactosyl transferases, dipeptidylamino peptidase IV, etc.), the ENOX2 proteins are shed, appearing in soluble form in conditioned media of cultured cells and in patient sera (Wilkinson et al., 1996; Morré et al., 1997). The ENOX2 transcript variants from sera exhibit the same degree of drug responsiveness as do the membrane-associated forms (Morré and Reust, 1997; Morré et al., 1997). With sera from more than 200 breast cancer patients, the majority (ca. 196), were found to exhibit the drug-responsive activity. In contrast, no drug-responsive activities were found with sera from healthy volunteers or sera from patients with diseases other than cancer (cardiac, arthritis and other inflammatory diseases, gastric ulceration, emphysema, various non-malignant blood disorders). As such the antitumor drug-responsive ENOX2 activities represent novel cell surface properties potentially associated with most, if not all, forms of human cancer to confirm their appropriateness as appropriate biomarkers for serum or plasma detection and diagnosis of cancer. ENOX2 proteins are robust and highly resistant to heat and protein degradation which enhances their utility as non-invasive markers for cancer detection and diagnosis (Morré and Morré, 2003a).

ENOX2 proteins are absent or present at levels below the limits of detection (less than 10 picomoles/ml of serum) from sera of healthy volunteers or patients with diseases other than cancer. Circulating ENOX2 has been detected based on drug response of ENOX activity of sera of more than 500 cancer patients representing all major forms of human cancer including leukemias and lymphomas (Morré and Reust, 1997; Morré et al., 1997).

4.3 Very early detection and diagnosis is unique to ENOX2 transcript variants

Many cancers are detected only after clinical symptoms present and often after the cancer has spread leaving chemotherapy as perhaps the only resource for treatment. Tomographic or x-ray methods may detect before clinical symptoms present but only after a tumor mass has already formed. There appear to be few, if any, on-going indications of opportunities either for early cancer detection or for early intervention. Various genomic, transcriptomic and/or proteomic analyses, while of potential utility for tissue analyses of biopsy material, have thus far failed to provide new and reliable non-invasive serum indicators of cancer occurrence (Goncalves and Bertucci, 2011) despite continued promise offered by circulating microRNAs (Wu et al., 2011). A relatively small percentage of all cancers can be attributed to predisposing genes such as *BRACA1*, *BRACA2* and less frequently *p53* and *PTEN* (Lee et al., 2010) for 5 to 10% of all breast cancers. While indicative of cancer risk, predisposing genes do not necessarily signal cancer presence.

4.4 Early intervention strategy based on green tea- *Capsicum* synergies

The potential benefits of early detection will not be fully realized without some opportunity for early curative or preventive intervention. As an early intervention strategy, findings suggest that a decaffeinated green tea extract containing 98% tea catechins of which 40% are EGCg and a *Capsicum* powder in the ratio of 25 parts tea extract plus 1 part *Capsicum* powder, available on line as Capsol-T (www.Capsol-T.com) and under commercial development by Stratum Nutrition, a division of Novus International, St. Charles, Mo under the brand name TeaFense may induce apoptosis as a means to eliminate early stage cancer when only a small number of cells are present prior to development of clinical symptoms. The ENOX proteins are responsible for the increase in cell size following cell division. After cell division, a minimum cell size must be reached or cell division stops and after several days, the cells undergo programmed cell death (apoptosis). Cancer cells with blocked ENOX2 activity are not able to enlarge and are thus directed towards apoptosis. The growth inhibition is due mainly to cell cycle arrest in G₁ (stage of cell division before DNA is replicated). EGCg inhibits growth of cancer cells in culture and in implanted tumors in mice (Li et al., 2010). The growth of implanted tumors was inhibited in a dose-dependent manner at doses of 0.1%, 0.3% and 0.5% in the diet.

Morré and Morré (2003b) have described synergy of decaffeinated green tea and a commercially available *Capsicum* preparations containing anti-cancer vanilloids (Capsibiol-T) at a ratio of 25:1 which resulted in a 100-fold increase in killing of cultured cancer cell lines compared to green tea alone. The current food grade *Capsicum*-green tea product (Capsol-T[®]) gives equivalent results. EGCg, when combined with other catechins also found in green tea, is superior to EGCg alone (Morré et al., 2003).

Evidence from laboratory studies with cancer cells in culture indicate that one 250 mg capsule of Capsol-T[®] every 4 h is equivalent to drinking 16 cups of green tea every 4 h. The need for 1 capsule of Capsol-T[®] every 4 h is predicated on pharmacokinetic information (Janle et al., 2008) and the knowledge that the inhibition of ENOX2 by Capsol-T[®] is reversible (Morré et al., 2000). In order to have therapeutic efficacy in selective killing of cancer cells, findings with cultured cancer cells show that the catechins must be present in the culture medium at a level of about 100 nM and to inhibit ENOX2 continuously at that level for a period of 48 to 72 h (Morré et al., 2000). If EGCg, for example, is removed and

replaced by EGCg-free media, even after 8 h, cancer cells in vivo resume normal rates of growth. Similarly, normal rates of growth are resumed as EGCg is cleared from the culture medium and/or metabolized. Even in cell culture, the EGCg may not survive in the media for more than a few h at nanomolar concentrations. The cancer cells in vitro must be inhibited from growing for at least 48 and perhaps up to 72 h in order for apoptosis to be induced by EGCg in a majority of the cancer cells present.

Feasibility of an efficacious dosing schedule is indicated from studies with rats (Janle et al., 2008). The results from the animal study are consistent with epidemiological studies in humans and animal experiments where cancer benefit has been ascribed to drinking at least 10 cups of green tea per day without adverse effects (Fujiki, 1999; Nakachi et al., 2000). Green tea polyphenols are absorbed after oral administration and reach their highest plasma levels after about 1 to 2 h after dosing both in rats (Unno and Takeo, 1995; Zhu et al., 2000; Janle et al., 2008) and in humans (Warden et al., 2001). In the rat, the levels of EGCg reached 12.3 nmoles/ml in plasma (12.3 μ molar) 60 min after a single oral administration of 500 mg/kg body weight of EGCg (Nakagawa, 1997), which is more than 100 times the effective dose to stop the growth of tumor cells. The studies by Yang (1997) show that the concentration of EGCg in the blood after 2-3 cups of green tea reached a maximum of about 0.6 μ M.

In human studies of ingested catechins, 0.2% of the ingested EGCg and 0.2% to 1.3% of ingested (-)-epigallocatechin (EGC) were found in plasma 90 min after ingestion (Nakagawa et al. 1997). Van het Hof et al. (1999) determined the half life for plasma levels of individuals drinking 8 cups of tea per day for 3 days to be 4.8 h for green tea and 6.9 h for black tea. After ingestion of green tea by human volunteers, C_{max} values were observed 1.4 to 2.4 h after injection with a half life of 5 to 5.5 h (Yang et al., 1998). These observations provided the rational basis for dosing at regular intervals of 4 h with the Capsol-T product. Formulated for sustained release, the expectation is that two 500 mg capsules of 50% material per day, one in the morning and one in the evening will prove to be sufficient.

Tea catechins especially EGCg in combination with *Capsicum* have been characterized as specific ENOX2 inhibitors inducing apoptotic cell death in cancer but not in non-cancer cells (Morré et al., 2000; Chueh et al., 2004; Morré et al., 2009a). Safety and efficacy are well documented (Cooper et al., 2005). Safety has been the subject of a series of reports dealing with genotoxic, acute, dermal, sub-chronic short-term, teratogenic and reproductive assays (e.g. Isbrucker et al., 2006a,b,c). Capsol-T[®] is both caffeine- and vitamin K-free and free of herbicide, pesticide and/or heavy metal residues. Tea as a food form is generally recognized as safe by the U.S. Food and Drug Administration (National Cancer Institute Fact Sheet).

Oral green tea extracts have been studied in human cancer patients. Pisters et al. (2001) did a phase 1 study with a commercially available but not decaffeinated green tea source given 1 or 3 times daily for 4 weeks to 6 months. Doses of 0.5 to 5.05 g/m² per day and 1.0-2.2 g/m² three times per day were tested in 49 cancer patients. The maximum tolerated dose of 4.22 g/m² was limited primarily by caffeine levels easily avoided with decaffeinated green tea.

In a series of open label sequential trials with Capsol-T[®] summarized by Morré and Morré (2006), 36% of participants with advanced cancer reported significant prolongation of life and/or remained alive at the time of the analysis. Another 32 reported improvement while the remaining 32% experienced a normal course of their disease. Most in the last category were diagnosed very late in the development of their disease such that an inability even to

comply with six capsule per day taken one every four hours became problematic. Preliminary human studies on patients (compassionate intervention) with severe head and neck carcinomas who were treated with a commercial preparation of the dietary supplement Capsibiol-T containing the mixture of decaffeinated green tea and modified chili peppers (*Capsicum* sp.), generated results that indicated a positive role for herbal mixtures of green tea and *Capsicum* for clinical use to eliminate cancer cells from the body (Fernandez et al., 2003).

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This unique synthesis of chapters from top experts in their fields targets the unique and significant area of cancer prevention for different types of cancers. Perspective readers are invited to go through novel ideas and current developments in the field of molecular mechanisms for cancer prevention, epidemiological studies, antioxidant therapies and diets, as well as clinical aspects and new advances in prognosis and avoidance of cancer. The primary target audience for the book includes PhD students, researchers, biologists, medical doctors and professionals who are interested in mechanistic studies on cancer prevention and translational benefits for optimized cancer treatment.

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