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Gene Section
Review

ENOX2 (ecto-NOX disulfide-thiol exchanger 2)

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Abstract

Review on ENOX2, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity

Other names: APK1, COVA1, tNOX

HGNC (Hugo): ENOX2

Location: Xq26.1

Note

Also termed APK1 antigen, or cytosolic ovarian carcinoma antigen 1, or tumor-associated hydroquinone oxidase (tNOX).
ECTO-NOX2 = Ecto-Nicotinamide Dinucleotide Oxidase Disulfide Thiol Exchange 2.

DNA/RNA

Description

The human ENOX2 gene is located on the reverse strand of chromosome X (bases 4918 to 284856); according to NCBI Refseq Gene Database (gene ID: 10495, RefSeq ID: NG_012562.1), and is comprised of 279939 bp.
ENOX2 is composed of 13 protein-coding exons between 71 bp and 2066 bp in length and 14 introns which vary greatly in length (1781 bp to 117994 bp).
It has a 501 bp 5' untranslated region and a long 3' UTR (approximately 1935 bp).

Transcription

According to NCBI the human ENOX2 gene encodes a 4036 bp mRNA transcript, the coding sequence (CDS) located from base pairs 356 to 2101 (NM_001281736.1).
The CDS from the Ensembl genome browser database (ENST00000370927, transcript length 3788 bp) and NCBI (NM_001281736.1) are identical.
Transcripts NM_001281736.1 and ENST 00000370927 are also included in the human CCDS set (CCDS14626) and encode a 610 aa long protein.

Pseudogene

None known.

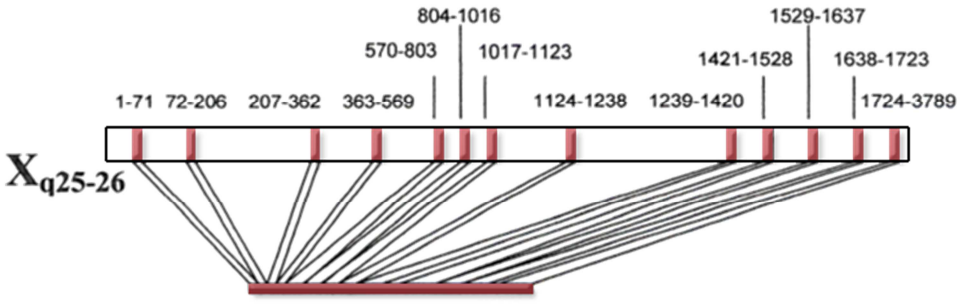

Figure 1. ENOX2 mRNA.

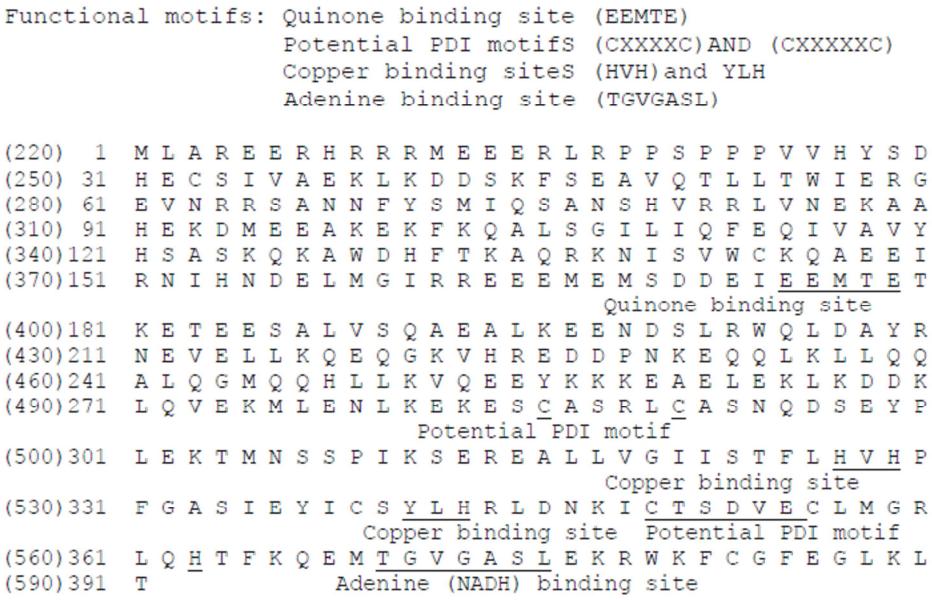

Figure 2. Deduced amino acid sequence and functional motifs of the bacterially expressed 46 kDa enzymatically active C-terminus of ENOX2.

Protein

Description

ENOX2 transcription variants all appear to be variations that include an exon 4 minus splicing event that allows for down-stream initiation and expression of the ENOX2 protein at the cell surface of malignant cells (Tang et al., 2007a; Tang et al., 2007b). Without the exon 4 deletion, mRNA derived from the gene does not appear to be translated into protein. Thus, the exon 4 deletion is the basis for the cancer specificity of the ENOX2 transcription variants. An hnRNP splicing factor directs formation of the Exon 4 minus variants of ENOX2 (Tang et al., 2011). The fully processed 34 kDa generic ENOX2 protein found on the cell surface of HeLa cells and in sera of about 23% of early cancer patients retains full-functional activity. The deduced amino acid sequence of a bacterially expressed 46 kDa functional C-terminus of ENOX2 exhibits the same characteristics of alternation of the two activities and drug response as the cell surface and generic serums forms. Identified functional motifs include a quinone binding site, an adenine nucleotide binding site, a CXXXXC cysteine motif as a potential disulfide-thiol interchange site and two copper binding sites, one of which is conserved with superoxide dismutase. ENOX2 proteins lack flavin and only one of the two C-X-X-X-X-C motifs characteristic of flavoproteins are present in ENOX2. Yet the protein effectively carries out protein disulfide interchange. The motif C569-X-X-X-X-X-C575, alone or together with a downstream histidine (H582) provides an additional potential active site for protein disulfide-thiol interchange (Morré and Morré, 2013).

The signature ENOX2 motif is that of the potential drug/antibody binding site E394EMTE. Antisera directed to this portion of the protein act as competitive inhibitors to drug binding. The sequence provides a putative quinone or sulfonylurea-binding site with four of the five amino acids in at least one other putative quinone site in the same relative positions.

The correctness of the various assignments has, for the most part, been confirmed by site-directed mutagenesis (Chueh et al., 2002).

While amino acid replacements that block oxidation of reduced pyridine nucleotide by ENOX2 also eliminated protein disulfide-thiol interchange and vice versa (Chueh et al., 2002), the two activities appear to occur independently.

One can be measured in the absence of the other.

The ENOX2 proteins have properties of prions and are protease resistant (Kelker et al., 2001) and N-terminal sequencing.

Concentrated solutions aggregate and form amyloid-like filaments.

Expression

Widely expressed in malignant cells but only as exon 4 minus splicing variants (Tang et al., 2007b).

Localisation

External cell surface (Morré, 1995).

Function

ENOX2 is a member of a family of cell surface metalocatalysts with binuclear copper centers that oscillate.

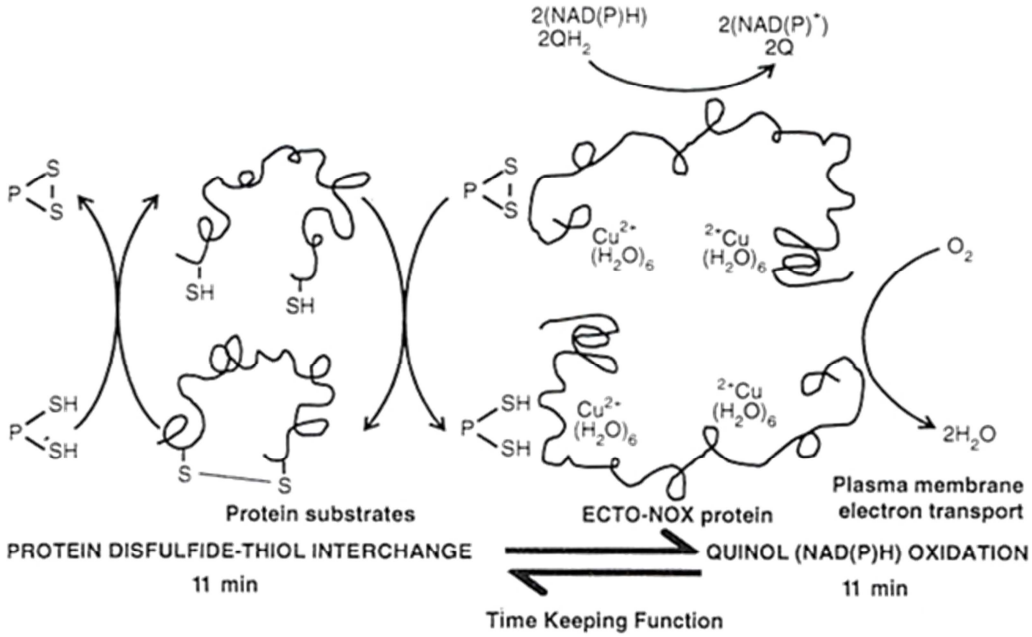

Figure 3. Diagrammatic representation of the functional unit of the ENOX2 proteins which is a dimer, each monomer of which contains two copper centers. During the oxidative portion of the ENOX cycle on the right, the net result is the transfer of 4 electrons plus 4 protons to molecular oxygen to from $2H_2O$. The left portion of the diagram illustrates the protein disulfide-thiol interchange activity portion of the cycle where the result is an interchange of protons and electrons that results in the breakage and formation of disulfide bonds important to cell enlargement.

They catalyze both NAD(P)H and hydroquinone oxidation in one configuration and carry out protein disulfide-thiol interchange in a second configuration (Figure 3). The two activities alternate creating a regular 22 min period to impart a time-keeping function (Morré and Morré, 2003). The oscillations are highly synchronous and phased by low frequency electromagnetic fields.

Functionally ENOX2 proteins of cancer cells act as terminal oxidases of plasma membrane electron transport (PMET) whereby electrons coming from cytosolic NAD(P)H are transferred to membrane-located coenzyme Q with eventual transfer of electrons and proteins to oxygen to form water (Figure 4). The released energy is presumably utilized to drive cell enlargement. The protein disulfide-thiol interchange part of the cycle carries out a function essential to the cell enlargement mechanism (Morré et al., 2006). The phenotype of unregulated accelerated growth is recapitulated in a transgenic mouse strain over expressing human ENOX2 (Yagiz et al., 2006).

Homology

RNA recognition motif (RRM) in the cell surface Ecto-NOX disulfide-thiol exchanger (ECTO-NOX or ENOX) proteins. This subgroup corresponds to the conserved RNA recognition motif (RRM) in ECTO-NOX proteins (also termed ENOX), comprising a family of plant and animal NAD(P)H oxidases exhibiting both oxidative and protein disulfide-like activities.

The ENOX2 gene is present in the human genome as a single copy, with no obvious homologs and a single constitutive ENOX1 (CNOX) ortholog with 64% identity and 80% similarity (Jiang et al., 2008).

Mutations

Somatic

No reports of mutations leading to inactivation of or inability to express ENOX2.

Implicated in

Various cancers

Note

The ENOX2 protein is universally associated with malignancies. It is not the result of an oncogenic mutation but appears to be similar if not identical to a form of ENOX protein with characteristics of an oncofetal protein important to maintenance of unregulated growth in very early development that may be re-expressed in malignancy (Cho and Morré, 2009). Re-expression as an oncofetal protein helps explain the role of ENOX2 of cancer cells in acquiring the well-known characteristic of uncontrolled growth. Consistent with this interpretation are observations that the malignant phenotypes of invasiveness and growth on soft agar of cancer cells in culture are lost when cells are transfected with ENOX2 antisense (Chueh et al., 2004; Tang et al., 2007a).

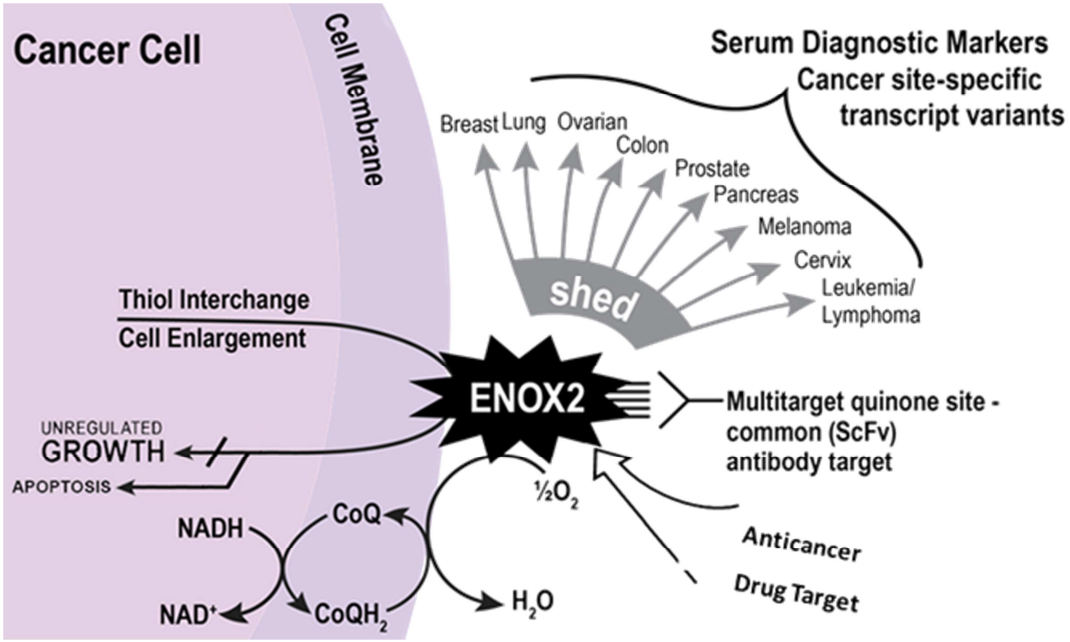

Figure 4. Schematic representation of the utility of the ENOX2 proteins as cancer-specific cell surface proteins for diagnosis and therapeutic intervention in cancer. Modified from Morré and Morré (2013).

ENOX2 is the first reported cell surface change absent from non-cancer cells and associated with most, if not all, forms of human cancer (Morré and Morré, 2013).

As such, ENOX2 emerges as a potential universal molecular cancer marker and, being an ecto-protein at the cell surface and shed into the circulation, a reliable cancer diagnostic marker both for cancer presence and tissue of cancer origin (Figure 4).

ENOX2 proteins are expressed differently by different tissues of cancer origin within the body with each type of cancer being characterized by one, two, three or more tissue specific transcript variants of characteristic molecular weights and isoelectric points (Morré and Morré, 2012). ENOX2 proteins are absent or reduced to below the limits of detection from sera of healthy individuals or patients with diseases other than cancer. Circulating ENOX2 has been detected in sera of patients representing all major forms of human cancer including leukemias and lymphomas.

All ENOX2 transcript variants appear to share the common antigenic determinant recognized both by an ENOX2-specific monoclonal antibody (Cho et al., 2002) and a corresponding scFv single chain variable region recombinant antibody expressed in bacteria and derived from the monoclonal antibody-producing hybridoma cells with analysis by 2-D-gel electrophoresis and western blot (Hostetler et al., 2009).

Breast cancer

Note

Sera of breast cancer patients contains an ENOX2 transcript variant of 64 to 69 kDa, isoelectric point 4.2 to 4.9.

Lung cancer

Note

Sera of patients with non-small cell lung cancer contain a 53 to 56 kDa ENOX2 transcript variant, isoelectric point pH 4.7 to 5.3 while sera of small cell lung cancer contain a transcript variant of 52 kDa, isoelectric point pH 4.1 to 4.6.

Prostate cancer

Note

Sera of patients with prostate cancer contain a 71 to 88 kDa ENOX2 transcript variant, isoelectric point pH 5.1 to 6.5.

Cervical cancer

Note

Sera of cervical cancer patients contain a 90 to 100 kDa transcript variant, isoelectric point pH 4.2 to 5.4.

Malignant melanoma

Note

Sera from malignant melanoma patients contain an ENOX2 transcript variant of 37 to 41 kDa,

isoelectric point pH 4.6 to 5.3.

Leukemias, lymphomas and myelomas

Note

Sera of patients with leukemia, lymphoma or myeloma, cancers having blood as the common tissue of origin, all contain ENOX2 transcript variants of 38 to 48 kDa and low isoelectric point pH 3.6 to 4.5.

Ovarian cancer

Note

Sera from ovarian carcinoma patients contain two ENOX2 transcript variants of 72 to 90 kDa and 37 to 47 kDa, both having similar isoelectric points in the range of pH 3.7 to 5.0.

Bladder cancer

Note

Sera of patients with carcinoma of the bladder contain two ENOX2 transcript variants of 63 to 66 kDa and 42 to 48 kDa with isoelectric points of 4.2 to 5.8 and 4.1 to 4.8, respectively.

Uterine cancer

Note

Sera of patients with uterine carcinoma contain two ENOX2 transcript variants of 64 to 69 kDa and 36 to 48 kDa with isoelectric points of pH 4.2 to 4.9 and pH 4.5 to 5.6.

Colorectal cancer

Note

Sera of patients with colorectal cancer contain at least two of three possible ENOX2 transcript variants of 80 to 96 kDa, isoelectric point pH 4.5 to 5.3, 50 to 60 kDa, isoelectric point pH 4.2 to 5.1 and 33 to 46 kDa, isoelectric point pH 3.8 to 5.2.

Other cancers

Note

Unique patterns of ENOX2 transcript variant expression (number, molecular with and isoelectric point) have been found as well associated with brain, endometrial, esophageal, gastric, hepatocellular renal cell, squamous cell, testicular germ cell and thyroid cancer as well as mesothelioma and sarcomas.

Endometriosis

Note

Invasive endometriosis is the only non-malignant disorder thus far characterized by the presence of unique ENOX2 transcript variants.

As a cancer therapeutic drug target

Note

ENOX2 is responsive to differentiating agents such as calcitriol and anticancer retinoids and inhibited by anticancer drugs such as doxorubicin, the anticancer sulfonylureas, the vanilloid capsaicin, the catechin EGCg and the cancer isoflavene phenoxodiol, all of which appear to function as quinone site inhibitors directed toward the EEMTE drug binding motif of ENOX2 (Morré and Morré, 2013; Hanau et al., 2014). The possibility of ENOX2 as a drug target is enhanced by the external location of the ENOX2 protein in a position to be readily available to drugs or antibodies conjugated to impermeant supports. As the growth involvement of ENOX2 proteins is in cell enlargement, ENOX2 inhibitors also block cell proliferation. The blocked cells, unable to enlarge, also fail to divide and eventually undergo apoptosis (Figure 4).

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