ARTICLE
Journal of Cellular Biochemistry 105:1437–1442 (2008)

Journal of Cellular Biochemistry

Cell Size Increased in Tissues From Transgenic Mice Overexpressing a Cell Surface Growth-Related and Cancer-Specific Hydroquinone Oxidase, tNOX, With Protein Disulfide-Thiol Interchange Activity

Kader Yagiz,[1] Paul W. Snyder,[2] D. James Morré,[3] and Dorothy M. Morré[1]*

[1]*Department of Foods and Nutrition, Purdue University, Stone Hall, 700 W. State Street, West Lafayette, Indiana 47907-2059*

[2]*Department of Veterinary Pathology, Purdue University, 625 Harrison Street, West Lafayette, Indiana 47907-2026*

[3]*Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, 201 S. University Street, West Lafayette, Indiana 47907-2064*

ABSTRACT

tNOX (ENOX2), a cancer-specific and growth-related cell surface protein with protein disulfide-thiol interchange and hydroquinone (NADH) oxidase activities was overexpressed in a transgenic mouse model. Female transgenic mice grew faster than wild type as did embryonic fibroblast cells prepared from the transgenic mice. The tissue expression of tNOX mRNA was greatest in heart, lung and liver. When these tissues were analyzed for cell size, the cells from the tissues of transgenic animals were, on average, 20% larger in surface area than cells from corresponding wild-type tissues. Also analyzed were cells of intestine, spleen and kidney in which tNOX overexpression was observed but to a lesser extent. Cell size was increased as well with intestine and kidney but less so with spleen. At the end of the study, carcass weights of the transgenic animals were greater than those of wild type. This increase in carcass weight was reflected in an increase in femur weight and thickness in both male and female transgenic mice but not in femur length. Other carcass parameters such as skin weight and body fat or body fluids were unchanged or changes were insufficient to account for the increased carcass weight. The findings are consistent with the property of tNOX observed in studies with cultured cells as contributing to the enlargement phase of cell growth. J. Cell. Biochem. 105: 1437–1442, 2008.

KEY WORDS: ECTO-NOX (ENOX); tNOX (ENOX2); NADH OXIDASE; TRANSGENIC MOUSE; (−)-EPIGALLOCATECHIN-3-GALLATE (EGCg); CELL ENLARGEMENT; GROWTH

Work in our laboratories has focused on a novel cancer-specific and growth-related cell-surface protein with protein disulfide-thiol interchange and hydroquinone (NADH) oxidase activities designated as tNOX tumor-associated NADH oxidase (GenBank Accession No. AF207881) [(ENOX2) Chueh et al., 2002]. It is a member of a family of cell surface (ECTO-NOX = ENOX) proteins and has been suggested to be the molecular target to explain the anticancer activity of the green tea polyphenol (−)-epigallocatechin-3-gallate (EGCg) [Morré et al., 2000; Chueh et al., 2004], the major catechin in green tea [Stoner and Mukhtar, 1995].

ENOX proteins function as terminal oxidases of plasma membrane electron transport to catalyze transfer of protons and electrons from cytosolic NAD(P)H via the membrane pool of reduced coenzyme Qs to molecular oxygen [Orczyk et al., 2005] or to protein disulfides [Chueh et al., 1997] as the terminal electron acceptors. Also a general feature of ENOX proteins is a protein disulfide-thiol interchange activity that correlates with cell enlargement [Morré, 1998]. Being ectoproteins, family members appear to be reversibly bound at the outer leaflet of the plasma membrane [Morré, 1995]. They are then shed from the cell surface and appear in soluble form in conditioned media of cultured cells [Morré et al., 1996] and in patient sera [Morré and Reust, 1997; Morré et al., 1997]. The oxidative activity of ENOX proteins is distinguished from that of other oxidases and oxidoreductases of both organelles and internal membranes and from other oxidoreductases of the plasma membrane by its resistance to inhibition by cyanide [Morré, 1998].

tNOX is both cancer specific and pancancer [Morré et al., 1995, 1997; Morré and Reust, 1997; Cho et al., 2002]. Cancer cells

*Correspondence to: Prof. Dorothy M. Morré, Department of Foods and Nutrition, 700 West State Street, Purdue University, West Lafayette, IN 47907-2059. E-mail: morredm@purdue.edu

Received 22 July 2008; Accepted 9 September 2008 • DOI 10.1002/jcb.21962 • 2008 Wiley-Liss, Inc.
Published online 3 November 2008 in Wiley InterScience (www.interscience.wiley.com).

expressing tNOX such as HeLa (human cervical carcinoma) and BT-20 (human mammary carcinoma) cells not only grow in an unregulated manner but their growth is inhibited by EGCg [Morré et al., 2000] and other quinone site inhibitors with anticancer activity [Morré, 1995, 1998; Morré et al., 1995, 1996, 1997; Morré and Reust, 1997; Cho et al., 2002]. When tNOX cDNA was overexpressed in COS or MCF-10A (non-cancer mammary epithelial) cells, the transfected cells exhibited an accelerated rate of cell enlargement, a larger cell size at confluence and a heightened susceptibility to growth inhibition by EGCg [Chueh et al., 2004].

Antisense results and the transfection and overexpression experiments taken together showed that functional cell surface expression of tNOX was both necessary and sufficient for the cancer-specific cell growth inhibitions attributed to EGCg [Chueh et al., 2004]. As an extension of these observations, transgenic animals overexpressing tNOX were generated and shown to grow faster than wild type. Moreover, the enhanced component of growth was sensitive to inhibition by EGCg. Similarly, mouse embryonic fibroblast (MEF) cells prepared from the tNOX overexpressing transgenic mice [Yagiz et al., 2006] exhibited both a cancer-specific and growth-related cell surface NADH oxidase with protein disulfide-thiol interchange activity and grew at rates approximately twice those of wild-type embryonic cells [Yagiz et al., 2007]. The growth of MEF cells from transgenic mice also was susceptible to inhibition by EGCg whereas the growth of wild-type cells was not. Specifically, rates of enlargement of the MEF cells from transgenic animals were accelerated compared to wild type so that the cells both divided faster and were larger at confluence. In this report, we measured cell diameters of fixed and stained tissues and other parameters which may contribute to increased carcass weight of the tNOX transgenic mice. The findings reveal increased cell size corresponding to increased organ weight attributed to the known phenotypic expression of tNOX which is to accelerate the enlargement phase of cell growth.

MATERIALS AND METHODS

MICE AND CELL LINES

The tNOX transgenic mice were generated as described [Yagiz et al., 2006]. Four transgenic female mice were bred with one transgenic male littermate and checked for vaginal plugs daily. Control FVB/N mice were bred in parallel.

TISSUE RT-PCR ANALYSIS

Total RNA was isolated from transgenic and wild-type tissues by using TRI-REAGENT (Molecular Research Center, Inc., Cincinnati, OH) reagents according to the manufacturer's instructions. The first strand cDNA was used as the template in the PCR reactions. The integrity of each tissue RNA sample was checked by RT-PCR with primers [5′-ACCCATACGATGTTCCGGATT-3′ (forward), and 5′-GGTCAGCTTCAAGCCTCGAAGC-3′ (reverse)]. Mouse GAPDH primers [5′-TCA CCA TCT TCC AGG AGC-3′ (forward), 5′-CTG CTT CAC CAC CTT CTT GA-3′ (reverse)] were used as internal standards. The negative control reactions included reagents without reverse transcriptase to ensure that the RT-PCR was RNA-dependent.

TISSUE ANALYSIS

To determine the weight differences of the tissues, transgenic and wild type of the same age mice were fasted overnight. The next day, the mice were weighed, killed and then the tissues were collected immediately. Weighed tissue samples were fixed in formalin (10% neutral buffered formaldehyde) solution (Fisher, Hampton, NH) and processed by the Purdue Histology and Phenotyping Laboratory (PHPL) at the School of Veterinary Medicine at Purdue University. The tissue samples were prepared for routine light microscopy analysis by the PHPL. Briefly, the fixed tissue samples were dehydrated gradually with 70%, 95%, and 100% ethanol solutions. The tissues were embedded in paraffin at 60°C for overnight. The next day, the paraffin blocks were cooled in the refrigerator for 20 min and then kept on ice preparatory to sectioning. The tissue sections (4–6 μm) were stained with Harris' Hematoxylin (Infolab, Inc., Zionsville, IN) and Eosin Y (0.5%) (Roboz, Gaithersburg, MD) solutions [Luna, 1968].

ORGAN WEIGHTS

Organ weights from transgenic and wild-type mice were compared. Six animals were examined in each group.

BONE ANALYSIS

The bone analysis was performed to investigate the weight differences between transgenic and wild-type mice. The right femur of individual transgenic and wild-type mice was used. Micro-CT (μCT) analysis was kindly provided by the laboratory of Professor Connie Weaver of the Foods and Nutrition Department at Purdue University using μCT-40 (Scanco Medical, Switzerland) to assess microstructural parameters and properties in cortical bone. One hundred slices from mid-shaft of the right femur were analyzed. The thickness of each slice was 6 μm. Besides the thickness of the cortical bone, the polar moment of inertia (pMOI) and the weight and length of the femur were analyzed. pMOI is a measure of the distribution of material around the center of a specimen. The following mathematical equation was used to calculate the pMOI: $\mathrm{pMOI} = \sum(d^2 \times A)\ (\mathrm{mm}^4)$. A is the cross-sectional area of voxel which is a volume element and represents a value in three dimensional space and d is the distance of the voxel from the center of gravity [Schoenau et al., 2006]. Femur weights were determined in parallel after physical removal of muscle and connective tissue following autoclaving.

MEASUREMENTS OF CELL SIZE

Measurements were from tissues collected from 3 to 10 transgenic animals and from 3 to 7 wild-type animals. Three 4–6 μm tissue sections were cut for each animal and the tissue types of interest were photographed at random.

Average cell area was determined by two methods. Average cell area is equivalent to the total area of a population of cells divided by the number of cells in that area. The first utilized the Glagolev–Chayes method of point counting [Glagolev, 1934; Chayes, 1956; Halgunset, 1984]. Cell areas were estimated for between 100 and 250 cells from each tissue section. Tabular values are aggregate analysis for each animal ± standard deviations among animals. For the second method, cross sectional areas were estimated by tracing each cell from digitally captured images. Areas were obtained in pixels

and then transferred into square micrometers by means of a stage micrometer. Cell volume (V) may be estimated from the area as follows: $r = \sqrt{A/N}$ and $V = (4/3)\pi r^3$.

STATISTICAL ANALYSIS

Statistical analyses were based on paired *t*-tests. Differences where treatments differed from controls by more than two standard deviations were highly significant. Individual *P* values are reported.

RESULTS

REVERSE TRANSCRIPTASE PCR

Comparing different tissue samples, the presence of the over-expressed tNOX gene was found by PCR analysis of tNOX mRNA in all tissues examined (heart, lung, liver, kidney, intestine, and spleen) of transgenic mice, but not in the comparable tissues of wild-type mice [Yagiz et al., 2006]. GAPDH transcripts were monitored as a control to quantify the transcripts of the genes in each tissue sample. Amounts of tNOX and GAPDH mRNAs were determined by densitometry, and ratios of tNOX/GAPDH mRNA were calculated. Compared to RT-PCR products using mouse GAPDH primers as internal controls, tNOX mRNA was expressed in heart - $\approx$ lung $\approx$ liver $\gg$ kidney $>$ intestine $\approx$ spleen (Fig. 1).

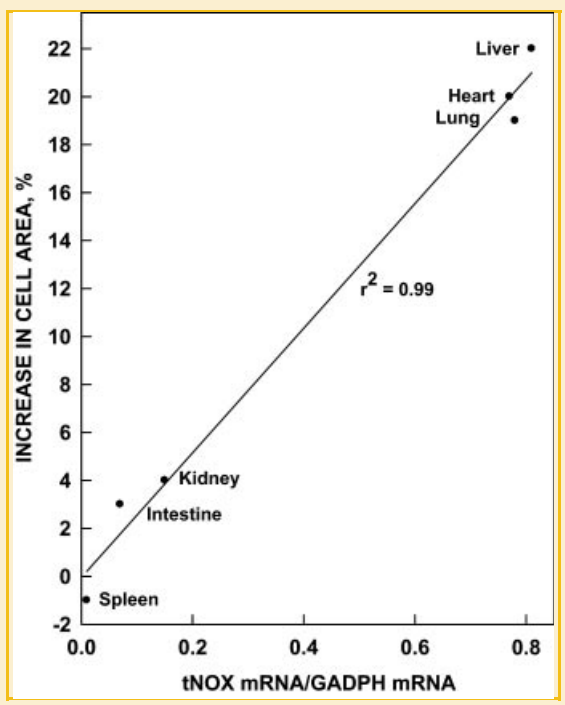

Fig. 1. Correlation between tissue expression of tNOX mRNA and increase in cell size in transgenic mice. Tissue samples were analyzed for tNOX mRNA by PCR. Quantitation was by densitometry and expressed as the ratio to glycer-aldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control [Yagiz et al., 2006]. Increases in cell size, in percent, are from Table III.

COMPARISON OF tNOX OVEREXPRESSION ON TISSUE MORPHOLOGY

Tissue samples of stomach, spleen, intestine, kidney, lung, heart, and liver from transgenic and wild-type mice revealed no gross morphological differences comparing tissues of transgenic and wild-type animals (not shown).

GROWTH AND ORGAN WEIGHT

The most obvious phenotypic characteristic of tNOX overexpression in the transgenic mice was an increased rate of growth resulting in larger animals. Between 3 and 9 months of age, the growth rate of transgenic mice was 25–50% faster than wild type [Yagiz et al., 2006; Table I] reflected in a 10–28% increase in carcass weight [Yagiz et al., 2006; Table I]. Also increased was overall organ weight (21–43%) (Table I).

According to data of Table I approximately 49% of the weight differential between female transgenic and wild-type mice was due to increased organ weights. Skin (19%) and carcass (32%) weight increases accounted for the remainder. With male mice, the bulk of the increase (59%) was in carcass weight with organs accounting equally for the remainder. The absolute increases in organ weight, 2.3 and 1.5 g for female and male mice, respectively, were more nearly equal.

Transgenic female mice reached a weight of wild-type male mice after 8–9 months of age. Even at 9 months of age, however, organ weights were still 14% greater for the transgenic mice compared to non-transgenic mice and were significantly increased by an average of 18% for heart, lung and liver. Smaller increase in weights for kidney, intestine, stomach and spleen averaging 11% were not statically significant (Table II). Similarly, organ weights were increased by 28% in male transgenic mice compared to wild type at 6 months of age with significant increases in weights of heart, liver and lung averaging 20% except for intestine which showed a 39% increase in transgenic mice compared to wild type (Table II).

Skin weights also were significantly greater for transgenic mice (Table I). Heads and brains, when weighed separately, were respectively, 6% and 18% larger in transgenic male and female mice compared to non-transgenic mice but the differences were not significant.

CELL AREA

The male animals at 6 months of age were selected for analysis of cell area (Table III). The average cell areas occupied by liver parenchyma cells, heart muscle cells and lung alveolar cells in tissues from transgenic mice were approximately 15–20% greater than in tissues from wild-type animals. These are the tissues that showed the highest levels of tNOX mRNA expression.

For intestinal absorptive cells, kidney cortical cell size from transgenic animals increased compared to wild type although differences were not significant statistically. Difference in size of splenocytes could not be discerned. Size of stomach cells was not analyzed due to the heterogeneity of the cell populations represented.

TABLE I. Tissue Weight Analysis Comparing Transgenic and Wild-Type Mice 6.5–7 Months of Age (n = 6)

	Females				Males			
	Wild type	Δ	Transgenic	% Increase	Wild type	Δ	Transgenic	% Increase
Weight (g)								
Total	22.3 ± 1.3	4.7	27.0 ± 0.7**	21	26.7 ± 1.6	6.9	33.6 ± 4.1*	26
Carcass	13.3 ± 0.5	1.5	14.8 ± 0.5**	11	14.8 ± 0.9	4.1	18.9 ± 1.7*	28
Skin	3.3 ± 0.5	0.9	4.2 ± 0.4*	27	4.2 ± 0.5	1.6	5.8 ± 0.4**	38
Organ	5.4 ± 0.4	2.3	7.7 ± 0.4**	43	7.0 ± 0.8	1.5	8.5 ± 0.4*	21

*Transgenic significantly different from wild type $P < 0.002$.
**Transgenic significantly different from wild type $P < 0.0001$.

BONE ANALYSES

Except for femur length, bone parameters were consistently increased in transgenic mice compared to wild-type mice (Table IV). Except for a 10–15% increase in femur weight, the differences observed were not statistically significant.

DISCUSSION

The ECTO-NOX proteins appear to be involved in the enlargement phase of cell growth. When overexpressed in COS cells, the tNOX cDNA resulted in larger cells and accelerated rate of cell enlargement [Chueh et al., 2004]. That the tNOX transgenic mice grew at an accelerated rate between 3 and 9 months of age up to 2-fold for females and 1.4-fold for males compared to wild-type mice was consistent with observations from tNOX transfected cells that tNOX facilitates the uncontrolled growth rate exhibited by cancer tissues and cell lines [Morré and Morré, 2003; Yagiz et al., 2007].

The increased growth rate of transgenic mice was reflected in an overall increased carcass weight. Although total skeletal mass was not determined, some of the increased carcass weight might have been due to this source (10–15% increase in femur weight with length being unchanged). Cortical thickness and polar moment of inertia as determined by MicroCT analyses were only slightly increased suggesting an overall increase in bone diameter as the basis for the increase in femur weight. Femur diameter of 6–8-month-old animals (n = 12) was increased by 19 ± 8% in transgenic males (6 months) and 8 ± 3% in transgenic females (8 months). Total skeletal mass was not determined.

Bone volume is normally determined by the relative rates of bone formation and bone resorption. While we did not specifically measure cell size in bone, an increase in average cell volume without an overall increase in cell number would account as well for the observed changes. Mechanical strains exerted by obesity have osteogenic effects on weight bearing bone sites (Schindler et al., 1972). However, since these animals were not obese, it may be that here the increased bone thickness contributed to rather than resulted from the increased body weight. There were contributions to overall body weight from fluid in females and from body fat in the males (Table III) but the females lacked extra fat and the males lacked fluid. The increase in carcass weight may have included increased bone mass as suggested by an overall increase in femur weight.

Implicit in the bone analyses is that the bulk of the increase in carcass weight was from increased muscle mass. Changes in cell size or cell length of smooth muscle cells are much more difficult to determine than for liver parenchyma, for example, and such measurements were not attempted.

For the most part, increases in organ weight and increases in cell size correlated with tNOX expression levels determined indepen-

TABLE II. Tissue Weight Analyses Comparing Wild-Type and Transgenic Mice

	Female			Male		
	Wild Type	Transgenic	% Increase	Wild Type	Transgenic	% Increase
Weight (g)						
Total body	21.8 ± 2.2	26.7 ± 1.6**	22	28.2 ± 1.9	33.0 ± 2.1**	17
Heart	0.13 ± 0.01	0.15 ± 0.02*	15	0.14 ± 0.02	0.17 ± 0.02*	21
Lung	0.31 ± 0.01	0.37 ± 0.02***	19	0.30 ± 0.05	0.36 ± 0.03*	20
Liver	1.21 ± 0.1	1.45 ± 0.05***	20	1.17 ± 0.12	1.4 ± 0.2*	20
Kidney	0.26 ± 0.03	0.27 ± 0.02	4	0.39 ± 0.05	0.42 ± 0.04	8
Intestine	2.0 ± 0.6	2.19 ± 0.22	10	2.8 ± 0.4	3.9 ± 0.4***	39
Stomach	0.35 ± 0.15	0.41 ± 0.08	17	0.48 ± 0.1	0.5 ± 0.1	4
Spleen	0.10 ± 0.01	0.12 ± 0.01	20	0.09 ± 0.1	0.11 ± 0.1	22
Carcass	12.0 ± 2.0	14.6 ± 1.0*	22	15.4 ± 1.0	19.8 ± 1**	29
Fat	–	–		1.0 ± 0.1	1.1 ± 0.5	
	2.7	2.99	11			
Fluid	0 ml	2 ml		–	–	
	4.36	4.96	14	5.37	6.86	28

Averages are ±standard deviations from six mice at age of 8 months for female mice and at age 6 months for male mice.
*Transgenic significantly different from wild type $P < 0.03$.
**Transgenic significantly different from wild type $P < 0.001$.
***Transgenic significantly different from wild type $P < 0.0001$.

TABLE III. Cell Area of Tissues of Male Mice Corresponding to Data of This Table Determined by Point Counting Method

	Cell area μ^2 (n = 4)		
Tissue	Wild Type	Transgenic	% Increase
Heart	545 ± 14	651 ± 46**	19
Lung	191 ± 14	229 ± 11**	20
Liver	503 ± 60	616 ± 16*	22
Kidney	278 ± 14	286 ± 10	3
Intestine	135 ± 5	140 ± 4	4
Spleen	132 ± 4	130 ± 3	−2

Results were based on analyses of four tissue sections from each of four mice ± standard deviations among individual mice.
*Transgenic significantly different from wild type $P < 0.01$.
**Transgenic significantly different from wild type $P < 0.005$.

dently (Fig. 1). tNOX expression was most evident in heart, lung and liver. These three organs also were increased in size significantly in the transgenic animals. For each of these tissues, cell size was also increased even after growth was nearly complete suggesting that the overall increase in organ mass was due to the established characteristic of tNOX overexpression resulting in an increase in cell size [Morré and Morré, 2003; Yagiz et al., 2007].

In animal growth, cells, once having divided, in order to divide again must enlarge to some minimal size required by the checkpoint in G_1 that monitors cell size. Except for the role of ENOX proteins, little is known about how cells enlarge or the enzyme systems involved. The ENOX family of cell surface copper-containing proteins with dual activities of hydroquinone oxidation and protein disulfide-thiol interchange appear to be essential to both animal and plant cell enlargement based on inhibitor and antibody studies [Morré, 1998]. When ENOX proteins are blocked or inhibited, cell enlargement is also blocked or inhibited. When ENOX proteins are stimulated or over expressed, cell enlargement also is accelerated [Chueh et al., 2004; Yagiz et al., 2006, 2007; Fig. 1]. The activity directly involved in the enlargement process emerges as that of the protein disulfide-thiol interchange activity [Morré and Morré, 2003]. Antisera to the human constitutive ENOX1 protein block both cell enlargement and protein disulfide-thiol interchange without effect on the rate of hydroquinone oxidase or oxidation of reduced pyridine nucleotides where NADH and NADPH serve as alternative substrates. Both activities are sensitive to impermeant thiol reagents further implicating the interchange activity in cell enlargement. The constitutive ENOX1 is highly regulated and responsive to growth factor and hormonal controls [Morré et al., 1998]. The cancer form, ENOX2, is constitutively activated and contributes to the cancer phenotype of unregulated cell growth [Morré and Morré, 2003]. Previous studies suggest that the mechanism is not one of passive addition of new material to the growing cell surface but an energy-requiring process of force-extension where the ENOX proteins act in cancert with an AAA-ATPase of the plasma membrane [Morré et al., 2006].

The tNOX transgenes were integrated randomly into the genome as multiple copies in a head-to-tail fashion. At some chromosomal locations, transgenes may be transcriptionally silent or disturb the functions of other genes. Therefore, the phenotypic characteristic of specific transgenic models depends on the function of the transgene as well as the insertion site in the chromosome. Since our transgene was introduced into the mouse genome randomly, we presently have no information on the location of its insertion in the genome. Even though, functional expression was evidenced by the presence of drug-inhibited NADH oxidase activity in the plasma membranes isolated from liver and intestine [Yagiz et al., 2006], and in the accelerated growth rates, especially in female mice inhibited by EGCg and the presence of an immunoreactive band corresponding to the processed molecular weight of tNOX in microsomes and plasma membranes of the several tissues examined [Yagiz et al., 2006].

Uncontrolled proliferation is a hallmark of cancer cells. Many cell cycle regulators that control the correct entry and progression through the cell cycle are altered in tumors. In fact, most, if not all, human cancers show a deregulated control of G1 phase progression, a period when cells decide whether to start proliferation or to stay quiescent [Golias et al., 2004]. That transgenic mouse embryo fibroblast (MEF) cells showed an accelerated rate of growth and cell enlargement compared to wild-type MEF cells could suggest possible differences in cell cycle. However, by flow cytometry, we observed that there was no difference in the cell cycle phases of transgenic and wild type MEF cell [Yagiz et al., 2007].

Similar alterations in cell size have been reported to result from manipulation of the PI3K/Akt pathway which may be partially explained by the ability of this pathway to regulate protein synthesis via downstream targets of the mammalian targets of rapamycin (mTOR), ribosomal protein p70 56-kinase and eukaryotic initiation factor 4E-binding protein-1/PHAS-I unrelated to cell cycle dysregulation [Faridi et al., 2003]. A relationship between tNOX overexpression and Akt activation remains to be investigated.

TABLE IV. Bone (femur) Parameters Comparing Transgenic and Wild-Type Mice

	Female			Male		
Femur	Wild Type	Transgenic	% Increase	Wild Type	Transgenic	% Increase
Weight (g)	0.039 ± 0.001	0.043 ± 0.002*	10	0.041 ± 0.002	0.047 ± 0.002**	15
Length (mm)	15.4 ± 0.6	15.3 ± 0.4	0	15.8 ± 0.3	15.6 ± 0.2	0
Cortical thickness (mm)	0.221 ± 0.005	0.224 ± 0.020	1	0.207 ± 0.008	0.218 ± 0.007	5
Polar moment of inertia (mm^4)	0.321 ± 0.060	0.329 ± 0.002	2	0.383 ± 0.043	0.409 ± 0.044	7

Values are means ± standard deviations of three mice per group.
*Transgenic significantly different from wild type $P < 0.04$.
**Transgenic significantly different from wild type $P < 0.02$.

ACKNOWLEDGMENTS

We thank Pramila Adhikara, Charles Kuntz, and Julie Moon for assistance with measurements.

REFERENCES

Chayes F. 1956. Petrographic model analysis. New York: Wiley. 113 p.

Cho N, Chueh PJ, Caldwell S, Morré DM, Morré DJ. 2002. Monoclonal antibody to a cancer-specific and drug-responsive hydroquinone (NADH) oxidase from the sera of cancer patients. Cancer Immunol Immunother 51:121–129.

Chueh PJ, Morré DM, Penel C, DeHahn T, Morré DJ. 1997. The hormone-responsive NADH oxidase of the plant plasma membrane has properties of a NADH: Protein disulfide reductase. J Biol Chem 272:11221–11227.

Chueh PJ, Kim C, Cho N, Morré DM, Morré DJ. 2002. Molecular cloning and characterization of a tumor-associated, growth-related, and time-keeping hydroquinone (NADH) oxidase (tNOX) of the HeLa cell surface. Biochemistry 41:3732–3741.

Chueh PJ, Wu LY, Morré DM, Morré DJ. 2004. tNOX is both necessary and sufficient as a cellular target for the anticancer actions of capsaicin and the green tea catechin (−)-epigallocatechin-3-gallate. BioFactors 20:235–249.

Faridi J, Fawcett J, Wang L, Roth RL. 2003. Akt promotes increased mammalian cell size by stimulating protein synthesis and inhibiting protein degradation. Am J Endocrinol Metab 285:E964–E972.

Glagolev AA. 1934. Quantitative analysis with microscopy by the ''point'' method. Eng Mining J 135:399–407.

Golias CH, Charalabopoulos A, Charalabopoulos K. 2004. Cell proliferation and cell cycle control: A mini review. Int J Clin Pract 12:1134–1141.

Halgunset J. 1984. Stereological estimation of average cell volume in monolayer culture by combined light and electron microscopy. J Microsc 135:325–336.

Luna GL. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology 3rd edition. New York: McGraw-Hill. pp. 9–25.

Morré DJ. 1995. NADH oxidase activity of HeLa plasma membranes inhibited by the antitumor sulfonylurea (N-4-methylphenylsulfonyl)-(N-4-chlorophenyl)urea (LY181984) at an external site. Biochim Biophys Acta 1240:201–208.

Morré DJ. 1998. NADH oxidase: A multifunctional ectoproteins of the eukaryotic cell surface. In Asard H, Bérczi A, Caubergs R, editors. Plasma membrane redox systems and their role in biological stress and disease. Dordrecht, The Netherlands: Kluwer Academic Publishers. pp. 121–156.

Morré DJ, Morré DM. 2003. Cell surface NADH oxidases (ECTO-NOX proteins) with roles in cancer, cellular time-keeping, growth, aging and neurodegenerative diseases. Free Radical Res 37:795–808.

Morré DJ, Reust T. 1997. A circulating form of NADH oxidase activity responsive to the antitumor sulfonylurea (N-4-methylphenylsulfonyl)-(N-4-chlorophenyl)urea (LY181984) specific to sera from cancer patients. J Bioenerg Biomembr 29:281–289.

Morré DJ, Chueh PJ, Morré DM. 1995. Capsaicin inhibits preferentially the NADH oxidase and growth of transformed cells in culture. Proc Natl Acad Sci USA 91:1831–1835.

Morré DJ, Wilkinson FE, Kim C, Cho N, Lawrence J, Morré DM, McClure D. 1996. Antitumor sulfonylurea-inhibited NADH oxidase of cultured Hela cells shed into media. Biochim Biophys Acta 1280:197–206.

Morré DJ, Caldwell S, Mayorga A, Wu LY, Morré DM. 1997. NADH oxidase activity from sera altered by capsaicin is widely distributed among cancer patients. Arch Biochem Biophys 342:224–230.

Morré DJ, Chueh PJ, Lawler J, Morré DM. 1998. The sulfonylurea-inhibited NADH oxidase activity of HeLa cell plasma membranes has properties of a protein disulfide-thiol oxidoreductases with protein disulfide-thiol interchange activity. J Bioenerg Biomembr 30:477–487.

Morré DJ, Bridge A, Wu LY, Morré DM. 2000. Preferential inhibition by (−)-epigallocatechin-3-gallate of the cell surface NADH oxidase and growth of transformed cells in culture. Biochem Pharmacol 60:937–946.

Morré DJ, Kim C, Hicks-Berger C. 2006. ATP-dependent and drug-inhibited vesicle enlargement reconstituted using synthetic lipids and recombinant proteins. Biofactors 28:105–117.

Orczyk J, Morré DM, Morré DJ. 2005. Periodic fluctuations in oxygen consumption comparing HeLa (cancer) and CHO (non-cancer) cells and response to external $(NADP)^{+}$/NAD(P)H. Mol Cell Biochem 273:161–167.

Schindler AE, Ebert A, Friedrich E. 1972. Conversion of androstenedione to estrone by human tissue. J Clin Endocrinol Metabolism 35:627–630.

Schoenau E, Neu CM, Rauch F, Manz F. 2006. The development of bone strength at the proximal radius during childhood and adolescence. J Clin Endocr Metabolism 86:613–618.

Stoner GD, Mukhtar H. 1995. Polyphenols as cancer chemopreventive agents. J Cell Biochem 22:169–180.

Yagiz K, Morré DJ, Morré DM. 2006. Transgenic mouse line overexpressing the cancer-specific tNOX protein has an enhanced growth and acquired drug-response phenotype. J Nutr Biochem 17:750–759.

Yagiz K, Wu L-Y, Kuntz CP, Morré DJ, Morré DM. 2007. Mouse embryonic fibroblast cells from transgenic mice overexpressing tNOX express an altered growth and drug response phenotype. J Cell Biochem 101:295–306.