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**Update on a tumor-associated NADH oxidase in gastric cancer cell growth**

Cheng HL *et al*. tNOX in gastric cancer cells

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**Abstract**

Gastric cancer is one of the most frequent human malignancies and has been shown to be well-correlated with cancer-related deaths worldwide. Regrettably, the poor prognosis of this disease is mainly due to the late diagnosis at advanced stage after the cancer has already metastasized. Recent research has emphasized the identification of cancer biomarkers in the hope of diagnosing cancer early and designing targeted therapies to reverse cancer progression. A member of a family of growth-related NADH (or hydroquinone) oxidases has been previously identified as a tumor-associated NADH oxidase (tNOX; ENOX2). Unlike its counterpart CNOX (ENOX1), identified in normal rat liver plasma membranes and shown to be stimulated by growth factors and hormones, tNOX activity purified from rat hepatoma cells, on the other hand, is constitutively active. The activity is also detected in the sera of cancer patients but not in those of healthy volunteers, suggesting its clinical relevance. Interestingly, tNOX expression is shown to be present in an array of cancer cell lines. More importantly, inhibition of tNOX is well correlated with reduced cancer cell growth and induction of apoptosis. RNA interference targeting tNOX expression in cancer cells effectively restores non-cancerous phenotypes, further supporting the vital role of tNOX in cancer cells. Here, we review the regulatory role of tNOX in gastric cancer cell growth.

**Key words:** Apoptosis; Capsaicin; Gastric cancer cells; Protein expression; Tumor-associated NADH oxidase

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**Core tip:** Gastric cancer is one of the most frequent human malignancies and has been shown to be well-correlated with cancer-related deaths worldwide. Here, we review the tumor-associated NADH oxidase tNOX (ENOX2), focusing on its role in the regulation of gastric cancer cell growth. The most common inhibitors of tNOX and the phenotypes associated with tNOX depletion are also discussed.

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**INTRODUCTION**

According to Global Cancer Statistics, nearly one million new gastric cancer cases and more than 700000 gastric cancer-associated death were reported in 2012[[1](#_ENREF_1)]. Unfortunately, despite progression in the diagnosis and treatment of advanced gastric cancer, many patients suffer from metastasis and later recurrence of this disease. Thus, their poor prognosis reflects the fact that gastric cancers are often diagnosed at an advanced stage. Current research efforts have focused on available diagnostic and prognostic biomarkers of gastric cancer. For example, carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA19-9) have been used as standard biomarkers for gastric cancer diagnosis[[2](#_ENREF_2)]. A method for detecting circulating tumor cells in gastric cancer patients that could provide important targets for treatment and critical surrogate markers also being developed[[3](#_ENREF_3)]. Moreover, polymorphisms of certain genes, such as epidermal growth factor receptor (EGFR), have been used as risk markers for gastric cancer[[4](#_ENREF_4)]. Another example is serum levels of interleukin-18 (IL-18), which plays a role in the pathogenesis of malignancies and is a determinant of clinical outcome in gastric cancer patients[[5](#_ENREF_5)]. In addition, recent studies have revealed that several microRNAs, when abnormally expressed, are potential biomarkers of gastric cancer[[6](#_ENREF_6)]. Although intensive efforts to identify biomarkers in this field are ongoing, universal biomarkers for gastric cancer are scarce because of the heterogeneous properties of this cancer[[2](#_ENREF_2)]. Thus, new and specific gastric cancer markers for diagnoses and therapeutic purpose are urgently needed.

We have previously described tumor-associated NADH oxidase (tNOX, also known as ENOX2), a member of a family of growth-related NADH (or hydroquinone) oxidases[[7-10](#_ENREF_7)]. Unlike its counterpart CNOX (ENOX1), identified in normal rat liver plasma membranes and shown to be stimulated by growth factors and hormones, tNOX activity purified from rat hepatoma cells is unresponsive to growth stimuli and is instead constitutively active[[7](#_ENREF_7)]. Subsequent studies have confirmed that tNOX is present in an array of cancer cell lines, including those derived from breast, cervix, colon, and lung cancer, as well as leukemias[[11-14](#_ENREF_11)]. The activity is also observed in the sera of cancer patients, but not in those of healthy volunteers, suggesting its clinical relevance[[15-17](#_ENREF_15)]. tNOX cDNA was subsequently cloned from a HeLa cell cDNA library[[9](#_ENREF_9)], and functional motifs of tNOX protein have been identified. The important role of tNOX in cell growth regulation is supported by the study that the growth rate of mouse embryo fibroblasts (MEFs) from tNOX-overexpressing transgenic mice is approximately twice that of wild-type cells[[18](#_ENREF_18)]. Given its expression in an array of cancer cell lines and presence in sera of cancer patients, taken together with other characteristics discussed below, tNOX possesses potential as a biomarker. Here, we briefly review the regulatory role of tNOX in gastric cancer cell growth.

**EXPRESSIONS OF tNOX PROTEIN IN GASTRIC CANCER CELLS**

Although tNOX expression has been demonstrated in an array of cancer cell lines, there is relatively less information regarding tNOX protein expression in gastric cancer cells. To demonstrate that tNOX protein is also expressed in gastric cancer cells, we utilized cell lines derived from human stomach cancers, including AGS (gastric adenocarcinoma), TMC-1 (derived from the lymph node of a moderately differentiated stomach adenocarcinoma), MKN45 (from the lymph node of a moderately differentiated stomach adenocarcinoma), SNU-1 (from a poorly differentiated primary carcinoma of the stomach), TMK-1 (from a poorly differentiated adenocarcinoma), and SCM (human gastric carcinoma) cells. Western blot analyses showed that tNOX protein is expressed to varying degrees in all gastric cancer lines (Figure 1). tNOX expression is higher in TMC-1, SCM and TMK-1 cells compared with SNU-1, MKN45 and AGS cells, where tNOX protein levels are somewhat lower. Antisera used in Western blot analyses were raised against bacterial tNOX as described previously[[12](#_ENREF_12)] and they recognize the same tNOX protein band as that identified by a purchased anti-tNOX polyclonal antibody commercially[[14](#_ENREF_14)] (Protein Tech Group, Inc. Chicago, IL, [United](javascript:void(0);) [States](javascript:void(0);)).

We also continuously monitored cell growth dynamics, measured as cell impedance and displayed in the form of cell index (CI) values[[19-22](#_ENREF_19)]. In this appliance of the cell impedance assay, the existence of cells on top of the electrodes creates an increase in electrode impedance whose degree is determined by cell numbers and the level of cell adhesion. By comparing the results from cell-impedance measurements with those obtained with various commonly used cytotoxicity assays, we have confirmed that this novel measurement of electrical impedance provides an effective, continuous-monitoring method for examining cellular processes in a dye-free setting[[23](#_ENREF_23),[24](#_ENREF_24)]. Using this approach, we found that both SCM and AGS cell grew much faster than MKN45 and TMC-1 cells (Figure 2). Surprisingly, the growth rate of TMC-1 cells determined using this method was slower, notwithstanding the fact that they were derived from patients with metastatic stomach cancer. It has been reported that TMC-1 cells grown *in vitro* exist as a mixture of attached and suspension cells[[25](#_ENREF_25)]. Thus, because the degree of cell impedance is decided by both cell numbers and the level of cell adhesions, the cell impedance method used here may yield a CI value that underestimates the number of TMC-1 cells.

**INHIBITION OF tNOX PROTEIN**

Numerous chemopreventative and anti-cancer drugs are demonstrated to reduce tNOX activity accompanied by a decrease in cell growth. These agents include capsaicin, the major component of chili pepper[[11](#_ENREF_11)], (-)-epigallocatechin-3-gallate (EGCg)[[26](#_ENREF_26)], phenoxodiol[[27](#_ENREF_27)], and doxorubicin (trade name, Adriamycin)[[28](#_ENREF_28),[29](#_ENREF_29)]. Interesting, these chemopreventive agents, often used to reverse cancer progression, preferentially inhibit tNOX activity in cancer cells, reducing cancer cell growth, but having little effect on non-cancerous cells[[11](#_ENREF_11), [26](#_ENREF_26)]. This is important since the dietary pattern and availability of fresh fruits and vegetables, as well as gastric cancer incidence, differ greatly among countries. Statistical data bear this out, showing that Korea, Mongolia, Japan, and China in Eastern Asia have the highest gastric cancer incidences, whereas Northern America and most parts of Africa have the lowest rate[[1](#_ENREF_1)]. Recent progress has focused on the chemopreventive effects of capsaicin, reflecting its anti-growth activity against various human cancer cell systems, including prostate[[30-32](#_ENREF_30)], colon[[33](#_ENREF_33),[34](#_ENREF_34)], hepatoma[[35](#_ENREF_35),[36](#_ENREF_36)], breast[[11](#_ENREF_11),[37](#_ENREF_37)] cancer, as well as leukemic[[38-40](#_ENREF_38)]. However, there are hardly any studies reporting the cytotoxicity of capsaicin in gastric cancer cells[[41-43](#_ENREF_41)].

We have investigated the effects of capsaicin on different gastric cancer cell line, including SCM, SNU-1 and TMC-1. In these studies, which measured metabolic activity by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assays, we verified that capsaicin exerts a concentration-dependent inhibitory effect on SCM cell proliferation. After 24-h exposure, 100 μmol/L and 200 μmol/L capsaicin decreased SCM cell viability to less than 70% and 50% of control groups, respectively. Cell proliferation measured by counting SCM cells numbers was also significantly decreased in a concentration and time-dependent manner by capsaicin exposure[[44](#_ENREF_44)]. We have also studied the anticancer activity of capsaicin on proliferation of SNU-1 cells which were derived from a poorly differentiated human gastric carcinoma, and TMC-1 cells, a metastatic gastric carcinoma line[[45](#_ENREF_45)]. These assays showed that capsaicin induces significant cytotoxicity in SNU-1 cells at 100 μmol/L, diminishing cell numbers to smaller than 40% and 30% of control groups after 48- and 72-h exposure, respectively. To our surprise, 100 μmol/L capsaicin did not decrease the number of TMC-1 cells, even after a 72-h exposure[[45](#_ENREF_45)]. These results imply that capsaicin exert differential cytotoxic effects on gastric cancer lines derived from different stages of cancer progression.

**DUEL EFFECTS OF CAPSAICIN ON CELL GROWTH AND tNOX IN TWO GASTRIC CANCER LINES**

To further investigate whether the differential inhibitory effects of capsaicin on cell growth inhibition involves cell death, specifically apoptosis, we analyzed cells for apoptotic subpopulations using flow cytometry. Interestingly, capsaicin provoked cytotoxicity in SCM cells concurrently with caspase 3-mediated PARP cleavage and apoptosis induction[[44](#_ENREF_44)]. Using the pan-caspase inhibitor Z-VAD-FMK, we confirmed that capsaicin-induced apoptosis in these cells is dependent on caspase activity[[44](#_ENREF_44)]. Consistent with results obtained in other gastric cancer cell lines, capsaicin was found to induce cytotoxicity and apoptosis in SNU-1 cells, possibly through up-regulation of p53[[42](#_ENREF_42)]. We also demonstrated that 100 and 200 μmol/L capsaicin triggered apoptosis in 16.1% and 26.2% of SNU-1 cells, respectively[[45](#_ENREF_45)]. In contrast, TMC-1 cells were fundamentally unresponsive to the apoptotic effect of capsaicin; exhibiting very little apoptosis in response to capsaicin exposure. The greater cytotoxicity of capsaicin toward SCM and SNU-1 cells was also reflected in the apoptotic activity of capsaicin in these two lines, whereas capsaicin induced neither cytotoxicity nor apoptosis in TMC-1 cells.

To identify molecular mechanisms underlying the differential cytotoxicity of capsaicin in gastric cancer cells, we assessed mitochondrial function, by measuring DiOC6(3) retention. We showed that capsaicin instigates changes in mitochondrial membrane potential in SNU-1 cells, however, not in TMC-1 cells[[45](#_ENREF_45)]. Further protein analyses have confirmed that capsaicin up-regulates pro-apoptotic Bak protein and down-regulates of anti-apoptosis Bcl-2 protein in SNU-1 cells, whereas these changes are less prominent in TMC-1 cells[[45](#_ENREF_45)].

Given that capsaicin-induced cytotoxicity and inhibition of tNOX activity have been previously established in cancer/transformed cells[[11](#_ENREF_11)], we next tested the possibility that tNOX protein is involved in the differential effects of capsaicin in these two gastric cancer lines. Interestingly, we found that capsaicin enhanced tNOX down-regulation in SCM cells concurrently with capsaicin-induced apoptosis[[44](#_ENREF_44)]. Additionally, in SNU-1 cells, capsaicin-induced tNOX down-regulation was accompanied by a concentration-dependent increase in caspase 3-mediated PARP cleavage and apoptosis[[45](#_ENREF_45)]. In contrast, capsaicin treatments promoted very slight changes in tNOX expression or caspase 3-directed PARP cleavage in TMC-1 cells, resulting in limited apoptosis. These various lines of evidences indicate that tNOX is a potential molecular target for capsaicin in gastric cancer cells that is important for gastric cancer survival.

**PHENOTYPES OF tNOX-DEPLETED CELLS**

To examine whether tNOX expression level is crucial for the survival of gastric cancer cells, we knocked down tNOX expression in TMC-1 cells using small interfering (hairpin) RNA (shRNA). These tNOX-depleted TMC-1 cells were more responsive to capsaicin treatments, as evidenced by enhanced caspase 3-mediated PARP cleavage, greater loss of mitochondrial membrane potential, and higher intracellular oxidative stress level compared with control groups[[45](#_ENREF_45)]. Thus, decreased tNOX expression in TMC-1 cells resulted in greater sensitivity to the effects of capsaicin. Interestingly, reduced expression of tNOX appeared to affect cell-cycle progression such that capsaicin-induced G1 accumulation was enhanced and proliferation was reduced in tNOX-depleted TMC-1 cells[[45](#_ENREF_45)], supporting a significant role for tNOX in TMC-1 cell growth.

We also examined the possibility that decreasing tNOX protein levels in cancer cells is sufficient to diminish tumor growth in animals. To this end, mice were injected with parental (wild type) HCT116 human colon cancer, or control (scrambled RNAi) HCT116 cells, and tNOX-knockdown HCT116 cells, and xenograft tumors were examined after 60 d. We found that the growth of HCT116 xenograft tumors was significantly reduced in tNOX-knockdown groups[[46](#_ENREF_46)], indicating that tNOX depletion in cancer cells reduces their capacity to form tumors *in vivo*.

The regulatory role of tNOX in cell growth is not limited in gastric cancer cells. Utilizing a loss-of-function approach, Chueh *et al*[[47](#_ENREF_47)] reduced tNOX expression in HeLa cervical cancer cells using antisense oligonucleotides and found that a tNOX deficiency reduces cell proliferation, as determined by colony-formation assays. A subsequent study utilizing shRNA to specifically and effectively inhibit tNOX expression in HeLa cells showed that tNOX knockdown attenuates cell proliferation and migration by interfering with the Rac pathway[[14](#_ENREF_14)]. In another example, tNOX-knockdown was shown to sensitize cells to stress-induced apoptosis in human HEK293 cells derived from human embryonic tissues[[13](#_ENREF_13)], which shares properties with cancer. Notably, we found that tNOX is abundantly expressed in these embryonic cells. Conversely, a gain-of-function approach has shown that tNOX overexpression in non-cancerous MCF-10A cells gives rise to enhanced invasiveness, an aggressive feature of cancer cells, confirming a vital role for tNOX in cancer progression[[47](#_ENREF_47)]. Moreover, transient up-regulation of tNOX in HCT116 cells augments cell proliferation and migration *in vitro* and *in vivo*[[46](#_ENREF_46)]. These phenomena are also observed in A549 human lung cancer cells, demonstrating that an epithelial-to-mesenchymal transition mechanism may be involved in the enhanced cell migration associated with tNOX up-regulation[[22](#_ENREF_22)].

CONCLUSION

In this review article, we have summarized the recent literature on the biological function of tNOX in gastric cancer cells. We also considered the paradoxical effect of capsaicin on cancer growth and tNOX expression that result in differential cellular outcomes. Collectively, these various lines of evidence establish a significant regulatory role for tNOX in cancer cell proliferation, survival, and migration. All the information may provide a reasonable framework for the future development of tNOX-targeting agents as a new class of anti-tumor therapeutics.

**References**

1 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]

2 **Jin Z**, Jiang W, Wang L. Biomarkers for gastric cancer: Progression in early diagnosis and prognosis (Review). *Oncol Lett* 2015; **9**: 1502-1508 [PMID: 25788990 DOI: 10.3892/ol.2015.2959]

3 [**Kolostova K**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kolostova%20K%5BAuthor%5D&cauthor=true&cauthor_uid=25862542), [Matkowski R](http://www.ncbi.nlm.nih.gov/pubmed/?term=Matkowski%20R%5BAuthor%5D&cauthor=true&cauthor_uid=25862542), [Gürlich R](http://www.ncbi.nlm.nih.gov/pubmed/?term=G%C3%BCrlich%20R%5BAuthor%5D&cauthor=true&cauthor_uid=25862542), [Grabowski K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Grabowski%20K%5BAuthor%5D&cauthor=true&cauthor_uid=25862542), [Soter K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Soter%20K%5BAuthor%5D&cauthor=true&cauthor_uid=25862542), [Lischke R](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lischke%20R%5BAuthor%5D&cauthor=true&cauthor_uid=25862542), [Schützner J](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sch%C3%BCtzner%20J%5BAuthor%5D&cauthor=true&cauthor_uid=25862542), [Bobek V](http://www.ncbi.nlm.nih.gov/pubmed/?term=Bobek%20V%5BAuthor%5D&cauthor=true&cauthor_uid=25862542). Detection and cultivation of circulating tumor cells in gastric cancer. *Cytotechnology* 2015; Epub ahead of print [PMID: 25862542 DOI: 10.1007/s10616-015-9866-9]

4 **Torres-Jasso JH**, Marín ME, Santiago-Luna E, Leoner JC, Torres J, Magaña-Torres MT, Perea FJ, Ibarra B, Sánchez-López JY. EGFR gene polymorphisms -216G& gt; T and -191C& gt; A are risk markers for gastric cancer in Mexican population. *Genet Mol Res* 2015; **14**: 1802-1807 [PMID: 25867325 DOI: 10.4238/2015.March.13.8]

5 **Tas F**, Tilgen Yasasever C, Karabulut S, Tastekin D, Duranyildiz D. Clinical significance of serum interleukin-18 (IL-18) levels in patients with gastric cancer. *Biomed Pharmacother* 2015; **70**: 19-23 [PMID: 25776473 DOI: 10.1016/j.biopha.2014.12.040]

6 **Liu HS**, Xiao HS. MicroRNAs as potential biomarkers for gastric cancer. *World J Gastroenterol* 2014; **20**: 12007-12017 [PMID: 25232237 DOI: 10.3748/wjg.v20.i34.12007]

7 **Bruno M**, Brightman AO, Lawrence J, Werderitsh D, Morré DM, Morre DJ. Stimulation of NADH oxidase activity from rat liver plasma membranes by growth factors and hormones is decreased or absent with hepatoma plasma membranes. *Biochem J* 1992; **284 (Pt 3)**: 625-628 [PMID: 1622384 DOI: 10.1042/bj2840625]

8 **Chueh PJ**. Cell membrane redox systems and transformation. *Antioxid Redox Signal* 2000; **2**: 177-187 [PMID: 11229524 DOI: 10.1089/ars.2000.2.2-177]

9 **Chueh PJ**, Kim C, Cho N, Morré DM, Morré DJ. Molecular cloning and characterization of a tumor-associated, growth-related, and time-keeping hydroquinone (NADH) oxidase (tNOX) of the HeLa cell surface. *Biochemistry* 2002; **41**: 3732-3741 [PMID: 11888291 DOI: 10.1021/bi012041t]

10 **Jiang Z**, Gorenstein NM, Morré DM, Morré DJ. Molecular cloning and characterization of a candidate human growth-related and time-keeping constitutive cell surface hydroquinone (NADH) oxidase. *Biochemistry* 2008; **47**: 14028-14038 [PMID: 19055324 DOI: 10.1021/bi801073p]

11 **Morré DJ**, Chueh PJ, Morré DM. Capsaicin inhibits preferentially the NADH oxidase and growth of transformed cells in culture. *Proc Natl Acad Sci USA* 1995; **92**: 1831-1835 [PMID: 7892186 DOI: 10.1073/pnas.92.6.1831]

12 **Chen CF**, Huang S, Liu SC, Chueh PJ. Effect of polyclonal antisera to recombinant tNOX protein on the growth of transformed cells. *Biofactors* 2006; **28**: 119-133 [PMID: 17379942 DOI: 10.1002/biof.5520280206]

13 **Mao LC**, Wang HM, Lin YY, Chang TK, Hsin YH, Chueh PJ. Stress-induced down-regulation of tumor-associated NADH oxidase during apoptosis in transformed cells. *FEBS Lett* 2008; **582**: 3445-3450 [PMID: 18789934 DOI: 10.1016/j.febslet.2008.09.008]

14 **Liu SC**, Yang JJ, Shao KN, Chueh PJ. RNA interference targeting tNOX attenuates cell migration via a mechanism that involves membrane association of Rac. *Biochem Biophys Res Commun* 2008; **365**: 672-677 [PMID: 18023414 DOI: 10.1016/j.bbrc.2007.11.025]

15 **Chueh PJ**, Morré DJ, Wilkinson FE, Gibson J, Morré DM. A 33.5-kDa heat- and protease-resistant NADH oxidase inhibited by capsaicin from sera of cancer patients. *Arch Biochem Biophys* 1997; **342**: 38-47 [PMID: 9185612 DOI: 10.1006/abbi.1997.9992]

16 **Morré DJ**, Caldwell S, Mayorga A, Wu LY, Morré DM. NADH oxidase activity from sera altered by capsaicin is widely distributed among cancer patients. *Arch Biochem Biophys* 1997; **342**: 224-230 [PMID: 9186482 DOI: 10.1006/abbi.1997.0110]

17 **Morré DJ**, Reust T. 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* 1997; **29**: 281-289 [PMID: 9298713 DOI: 10.1023/A:1022466212083]

18 **Yagiz K**, Wu LY, Kuntz CP, James Morré D, Morré DM. Mouse embryonic fibroblast cells from transgenic mice overexpressing tNOX exhibit an altered growth and drug response phenotype. *J Cell Biochem* 2007; **101**: 295-306 [PMID: 17115410 DOI: 10.1002/jcb.21184]

19 **Ke N**, Wang X, Xu X, Abassi YA. The xCELLigence system for real-time and label-free monitoring of cell viability. *Methods Mol Biol* 2011; **740**: 33-43 [PMID: 21468966 DOI: 10.1007/978-1-61779-108-6\_6]

20 **Moela P**, Choene MM, Motadi LR. Silencing RBBP6 (Retinoblastoma Binding Protein 6) sensitises breast cancer cells MCF7 to staurosporine and camptothecin-induced cell death. *Immunobiology* 2014; **219**: 593-601 [PMID: 24703106 DOI: 10.1016/j.imbio.2014.03.002]

21 **Kuo YF**, Su YZ, Tseng YH, Wang SY, Wang HM, Chueh PJ. Flavokawain B, a novel chalcone from Alpinia pricei Hayata with potent apoptotic activity: Involvement of ROS and GADD153 upstream of mitochondria-dependent apoptosis in HCT116 cells. *Free Radic Biol Med* 2010; **49**: 214-226 [PMID: 20398749 DOI: 10.1016/j.freeradbiomed.2010.04.005]

22 **Su YC**, Lin YH, Zeng ZM, Shao KN, Chueh PJ. Chemotherapeutic agents enhance cell migration and epithelial-to-mesenchymal transition through transient up-regulation of tNOX (ENOX2) protein. *Biochim Biophys Acta* 2012; **1820**: 1744-1752 [PMID: 22846226 DOI: 10.1016/j.bbagen.2012.07.009]

23 **Chuang SM**, Lee YH, Liang RY, Roam GD, Zeng ZM, Tu HF, Wang SK, Chueh PJ. Extensive evaluations of the cytotoxic effects of gold nanoparticles. *Biochim Biophys Acta* 2013; **1830**: 4960-4973 [PMID: 23811345 DOI: 10.1016/j.bbagen.2013.06.025]

24 **Chueh PJ**, Liang RY, Lee YH, Zeng ZM, Chuang SM. Differential cytotoxic effects of gold nanoparticles in different mammalian cell lines. *J Hazard Mater* 2014; **264**: 303-312 [PMID: 24316248 DOI: 10.1016/j.jhazmat.2013.11.031]

25 **Shyu RY**, Jiang SY, Jong YJ, Cheng KC, Lin CH, Yu JC, Wu MF, Chang TM. Establishment and characterization of a human gastric carcinoma cell line TMC-1. *Cells Tissues Organs* 2004; **177**: 37-46 [PMID: 15237194 DOI: 10.1159/000078426]

26 **Morré DJ**, Bridge A, Wu LY, Morré DM. Preferential inhibition by (-)-epigallocatechin-3-gallate of the cell surface NADH oxidase and growth of transformed cells in culture. *Biochem Pharmacol* 2000; **60**: 937-946 [PMID: 10974202 DOI: 10.1016/S0006-2952(00)00426-3]

27 **Morré DJ**, Chueh PJ, Yagiz K, Balicki A, Kim C, Morré DM. ECTO-NOX target for the anticancer isoflavene phenoxodiol. *Oncol Res* 2007; **16**: 299-312 [PMID: 17518268]

28 **Morré DJ**, Kim C, Paulik M, Morré DM, Faulk WP. Is the drug-responsive NADH oxidase of the cancer cell plasma membrane a molecular target for adriamycin? *J Bioenerg Biomembr* 1997; **29**: 269-280 [PMID: 9298712 DOI: 10.1023/A:1022414228013]

29 **Hedges KL**, Morré DM, Wu LY, Morre DJ. Adriamycin tolerance in human mesothelioma lines and cell surface NADH oxidase. *Life Sci* 2003; **73**: 1189-1198 [PMID: 12818726 DOI: 10.1016/S0024-3205(03)00421-1]

30 **Mori A**, Lehmann S, O'Kelly J, Kumagai T, Desmond JC, Pervan M, McBride WH, Kizaki M, Koeffler HP. Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. *Cancer Res* 2006; **66**: 3222-3229 [PMID: 16540674 DOI: 10.1158/0008-5472.CAN-05-0087]

31 **Sánchez AM**, Sánchez MG, Malagarie-Cazenave S, Olea N, Díaz-Laviada I. Induction of apoptosis in prostate tumor PC-3 cells and inhibition of xenograft prostate tumor growth by the vanilloid capsaicin. *Apoptosis* 2006; **11**: 89-99 [PMID: 16374544 DOI: 10.1007/s10495-005-3275-z]

32 **Sánchez AM**, Malagarie-Cazenave S, Olea N, Vara D, Chiloeches A, Díaz-Laviada I. Apoptosis induced by capsaicin in prostate PC-3 cells involves ceramide accumulation, neutral sphingomyelinase, and JNK activation. *Apoptosis* 2007; **12**: 2013-2024 [PMID: 17828457 DOI: 10.1007/s10495-007-0119-z]

33 **Kim CS**, Park WH, Park JY, Kang JH, Kim MO, Kawada T, Yoo H, Han IS, Yu R. Capsaicin, a spicy component of hot pepper, induces apoptosis by activation of the peroxisome proliferator-activated receptor gamma in HT-29 human colon cancer cells. *J Med Food* 2004; **7**: 267-273 [PMID: 15383218 DOI: 10.1089/jmf.2004.7.267]

34 **Kim YM**, Hwang JT, Kwak DW, Lee YK, Park OJ. Involvement of AMPK signaling cascade in capsaicin-induced apoptosis of HT-29 colon cancer cells. *Ann N Y Acad Sci* 2007; **1095**: 496-503 [PMID: 17404062 DOI: 10.1196/annals.1397.053]

35 **Baek YM**, Hwang HJ, Kim SW, Hwang HS, Lee SH, Kim JA, Yun JW. A comparative proteomic analysis for capsaicin-induced apoptosis between human hepatocarcinoma (HepG2) and human neuroblastoma (SK-N-SH) cells. *Proteomics* 2008; **8**: 4748-4767 [PMID: 18991268 DOI: 10.1002/pmic.200800094]

36 **Lee YS**, Kang YS, Lee JS, Nicolova S, Kim JA. Involvement of NADPH oxidase-mediated generation of reactive oxygen species in the apototic cell death by capsaicin in HepG2 human hepatoma cells. *Free Radic Res* 2004; **38**: 405-412 [PMID: 15190937 DOI: 10.1080/10715760410001665262]

37 **Kang HJ**, Soh Y, Kim MS, Lee EJ, Surh YJ, Kim HR, Kim SH, Moon A. Roles of JNK-1 and p38 in selective induction of apoptosis by capsaicin in ras-transformed human breast epithelial cells. *Int J Cancer* 2003; **103**: 475-482 [PMID: 12478662 DOI: 10.1002/ijc.10855]

38 **Ito K**, Nakazato T, Yamato K, Miyakawa Y, Yamada T, Hozumi N, Segawa K, Ikeda Y, Kizaki M. Induction of apoptosis in leukemic cells by homovanillic acid derivative, capsaicin, through oxidative stress: implication of phosphorylation of p53 at Ser-15 residue by reactive oxygen species. *Cancer Res* 2004; **64**: 1071-1078 [PMID: 14871840 DOI: 10.1158/0008-5472.CAN-03-1670]

39 **Lawen A**, Martinus RD, McMullen GL, Nagley P, Vaillant F, Wolvetang EJ, Linnane AW. The universality of bioenergetic disease: the role of mitochondrial mutation and the putative inter-relationship between mitochondria and plasma membrane NADH oxidoreductase. *Mol Aspects Med* 1994; **15 Suppl**: s13-s27 [PMID: 7752823 DOI: 10.1016/0098-2997(94)90009-4]

40 **Wolvetang EJ**, Larm JA, Moutsoulas P, Lawen A. Apoptosis induced by inhibitors of the plasma membrane NADH-oxidase involves Bcl-2 and calcineurin. *Cell Growth Differ* 1996; **7**: 1315-1325 [PMID: 8891335]

41 **Chow J**, Norng M, Zhang J, Chai J. TRPV6 mediates capsaicin-induced apoptosis in gastric cancer cells--Mechanisms behind a possible new "hot" cancer treatment. *Biochim Biophys Acta* 2007; **1773**: 565-576 [PMID: 17292493 DOI: 10.1016/j.bbamcr.2007.01.001]

42 **Kim JD**, Kim JM, Pyo JO, Kim SY, Kim BS, Yu R, Han IS. Capsaicin can alter the expression of tumor forming-related genes which might be followed by induction of apoptosis of a Korean stomach cancer cell line, SNU-1. *Cancer Lett* 1997; **120**: 235-241 [PMID: 9461043 DOI: 10.1016/S0304-3835(97)00321-2]

43 **Lo YC**, Yang YC, Wu IC, Kuo FC, Liu CM, Wang HW, Kuo CH, Wu JY, Wu DC. Capsaicin-induced cell death in a human gastric adenocarcinoma cell line. *World J Gastroenterol* 2005; **11**: 6254-6257 [PMID: 16419151]

44 **Wang HM**, Chueh PJ, Chang SP, Yang CL, Shao KN. Effect of Ccapsaicin on tNOX (ENOX2) protein expression in stomach cancer cells. *Biofactors* 2008; **34**: 209-217 [PMID: 19734122 DOI: 10.1002/biof.5520340305]

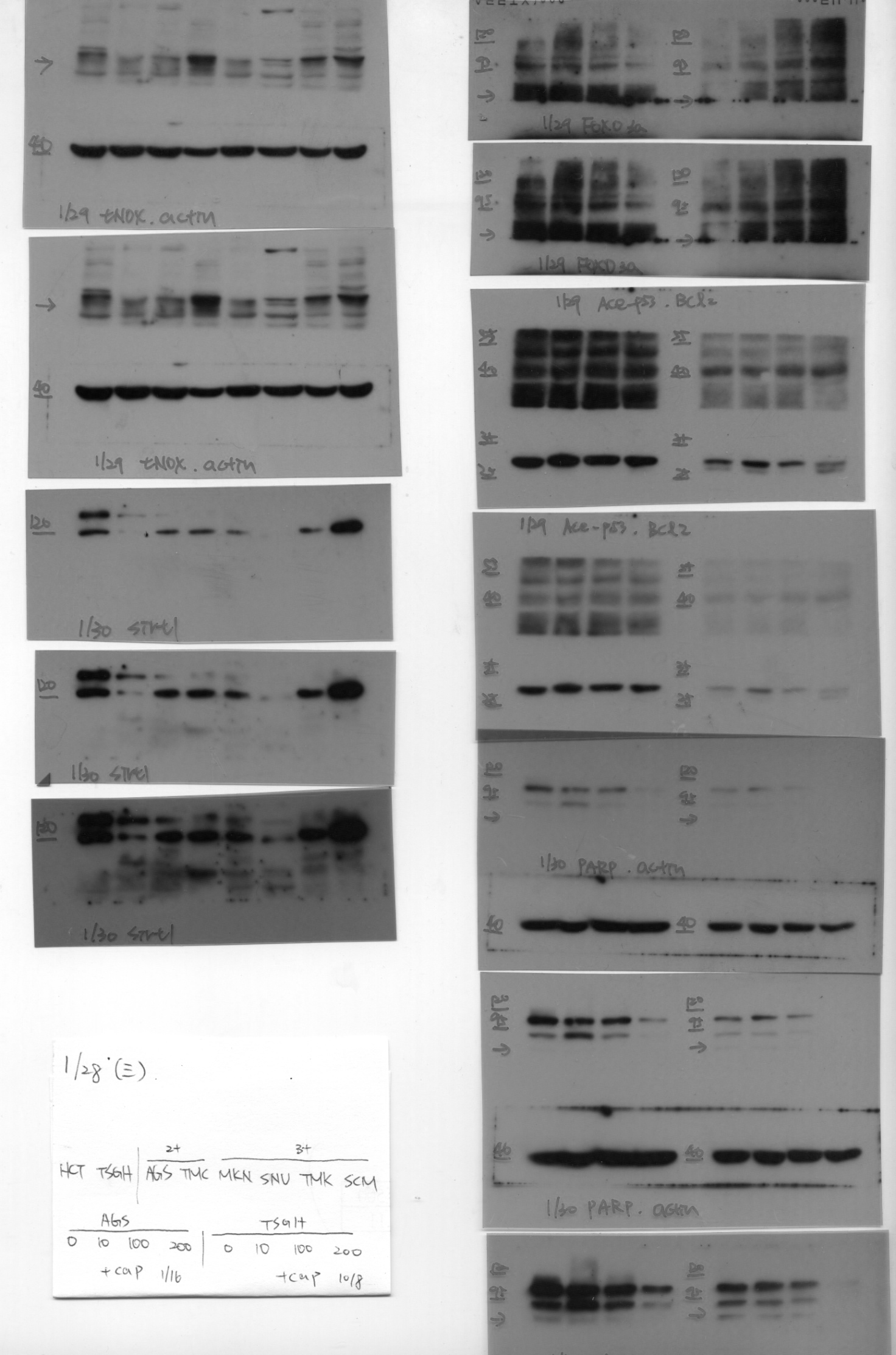
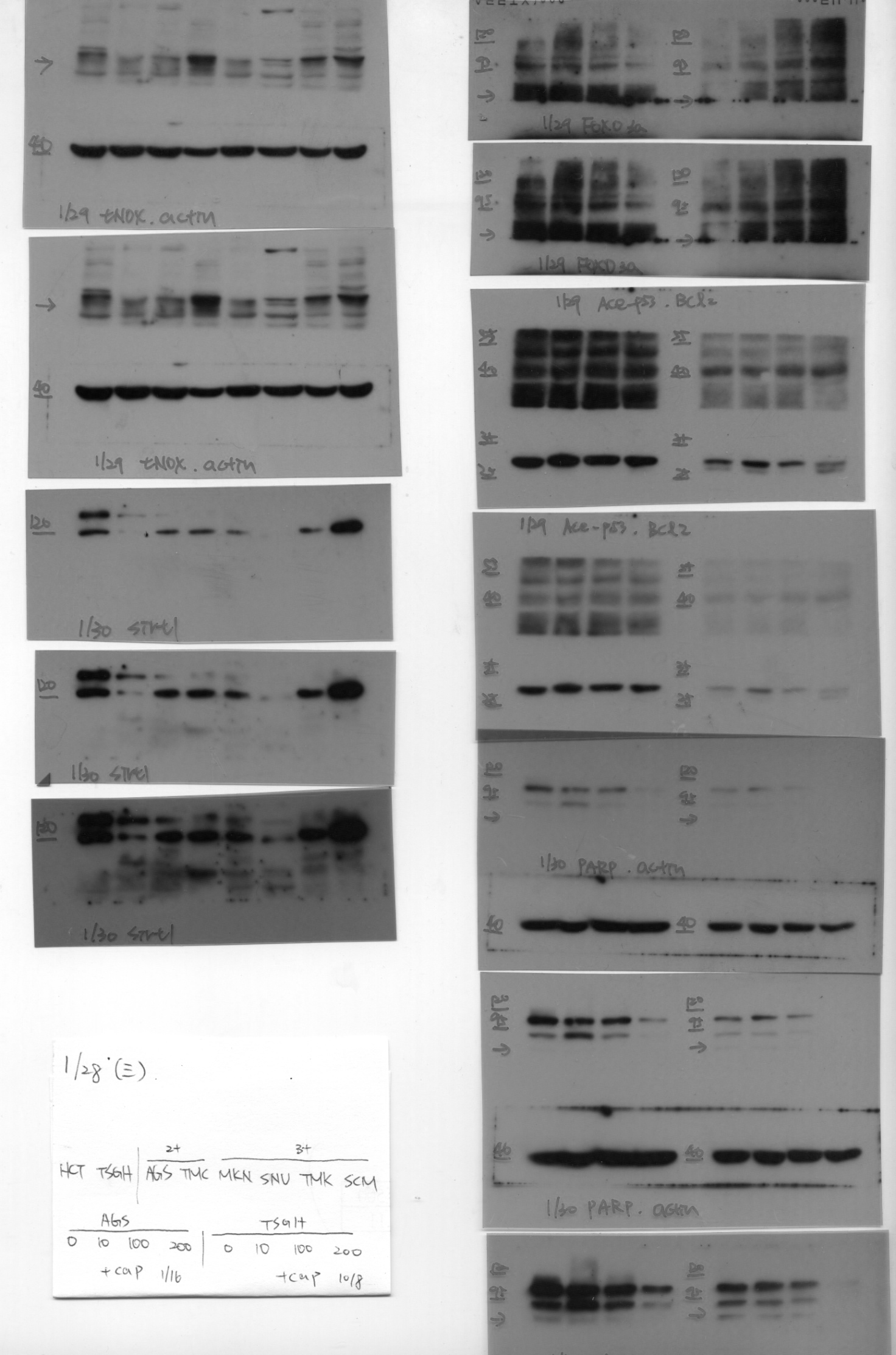
45 **Wang HM**, Chuang SM, Su YC, Li YH, Chueh PJ. Down-regulation of tumor-associated NADH oxidase, tNOX (ENOX2), enhances capsaicin-induced inhibition of gastric cancer cell growth. *Cell Biochem Biophys* 2011; **61**: 355-366 [PMID: 21735133 DOI: 10.1007/s12013-011-9218-0]

46 **Liu NC**, Hsieh PF, Hsieh MK, Zeng ZM, Cheng HL, Liao JW, Chueh PJ. Capsaicin-mediated tNOX (ENOX2) up-regulation enhances cell proliferation and migration in vitro and in vivo. *J Agric Food Chem* 2012; **60**: 2758-2765 [PMID: 22353011 DOI: 10.1021/jf204869w]

47 **Chueh PJ**, Wu LY, Morré DM, Morré DJ. 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* 2004; **20**: 235-249 [PMID: 15706060]

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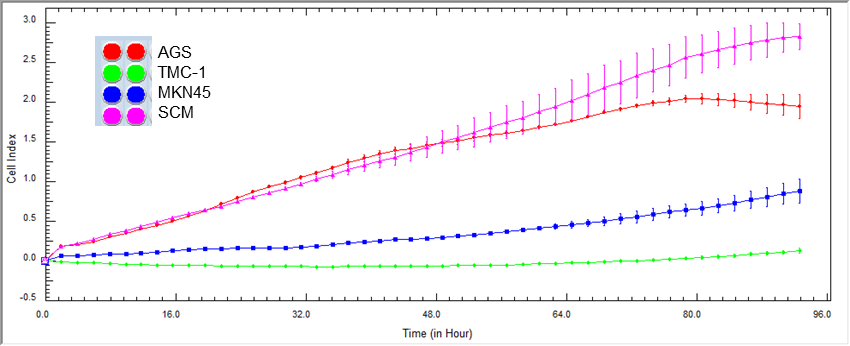


tNOX

Actin

AGS TMC-1 MKN45 SNU-1 TMK-1 SCM

**Figure 1 tumor-associated NADH oxidase protein expressions in different human gastric cancer lines.** Aliquots of cell lysates were separated by SDS-PAGE and analyzed for tNOX expression by Western blot analysis. β-Actin was used as an internal control to monitor for equal loading. Gastric cancer cell lines including AGS (gastric adenocarcinoma), TMC-1 (derived from the lymph node of a moderately differentiated stomach adenocarcinoma), MKN45 (from the lymph node of a moderately differentiated stomach adenocarcinoma), SNU-1 (from a poorly differentiated primary carcinoma of the stomach), TMK-1 (from a poorly differentiated adenocarcinoma), and SCM (human gastric carcinoma) were used to study the expression of tNOX protein. tNOX: tumor-associated NADH oxidase.

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**Figure 2 Cell growth monitored by cell impedance technology.** For continuous monitoring of dynamic changes in cell proliferation, cells (104 cells/well) were seeded onto E-plates and incubated for 30 min at room temperature, after which E-plates were positioned onto the Real-Time Cell Analysis station (Roche, Germany). Cells were grown and impedance was determined every hour, as previously described[[21](#_ENREF_21),[22](#_ENREF_22)]. Cell impedance is defined by the cell index (CI) = (Zi − Z0) [Ohm]/15[Ohm], where Z0 is background resistance and Zi is the resistance at an individual time point. A normalized cell index was determined as the cell index at a certain time point (CIti) divided by the cell index at the normalization time point (CInml\_time). Normalized cell index values measured over 92 h are shown. Gastric cancer cell lines including AGS (gastric adenocarcinoma), TMC-1 (derived from the lymph node of a moderately differentiated stomach adenocarcinoma), MKN45 (from the lymph node of a moderately differentiated stomach adenocarcinoma), and SCM (human gastric carcinoma) were used to study cell growth pattern using cell impedance measurements.