Minireview

Antioxidant enzymes and redox regulating thiol proteins in malignancies of human lung

Vuokko L. Kinnula[a,*], Paavo Pääkkö[b], Ylermi Soini[b]

[a]*Department of Medicine, Division of Pulmonary Diseases, University of Helsinki and Helsinki University Hospital, P.O. Box 22 (Haartmaninkatu 4), 00014 Helsinki, Finland*
[b]*Department of Pathology, University of Oulu and Oulu University Hospital, Oulu, Finland*

Received 18 March 2004; revised 6 May 2004; accepted 25 May 2004

Available online 7 June 2004

Edited by Vladimir Skulachev

Abstract Oxidants are known to modulate cell proliferation and apoptosis, and induce synthesis of growth factors that play an important role in tumor growth and invasion. Antioxidant enzymes and thiol proteins regulating cellular redox state constitute the major cellular protection against oxidants. Consequently, they are also associated both with carcinogenesis and tumor progression. Superoxide dismutases, glutamate cysteine ligase, catalase, thioredoxins and peroxiredoxins, which are the most important of these enzymes, are expressed in lung malignancies, and especially in pleural mesothelioma. This has consequences not only for tumor behavior but also for resistance of tumor cells to cytotoxic drugs and radiation.

Keywords: Reactive oxygen species; Malignancy; Cancer; Redox; Antioxidant

1. Introduction

The lung is directly exposed to higher levels of oxygen than most other tissues. The level of reactive oxygen species (ROS) in the lung is further increased by cigarette smoke, inflammation, pollutants, chemicals and carcinogens, that lead to DNA damage also by a free radical related mechanism [1–3]. Oxidants, imbalance between the cellular redox state and pulmonary defense systems evidently play a role both in the pathogenesis and in the progression of malignant lung diseases. Lung cancer that is highly associated with cigarette smoking is the most common malignancy worldwide, and its incidence is still increasing. Pleural mesothelioma is associated with asbestos, potent generator of free radicals, in most of the cases. Mesothelioma is uncommon, but also its incidence is increasing, the peak of its epidemic is expected in 2020 [4]. A common feature in both these malignancies is their poor prognosis and high resistance to chemotherapies and radiation.

There is clear evidence that free radicals are linked both to carcinogenesis and tumor behavior. Most important reactive metabolites also in human lung include superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\cdot$), and a number of reactive nitrogen and sulphur species (RNS, RSS) (peroxynitrite, oxidized states of sulfur) which are generated by multiple enzymatic reactions including NADPH oxidase ($NADPH_{ox}$), peroxidases and inducible nitric oxide (NO) synthase (Fig. 1). One major hypothesis explaining the importance of oxidants and imbalance of the cellular redox state in lung carcinogenesis is altered pro-oxidant intracellular environment that facilitates mutations and/or inactivation of tumor suppression genes and activates oncogenes with consequent changes in cell growth, survival and apoptosis [3,5,6]. Cellular redox state regulates several pathways closely associated with cell growth and survival including cMyc-, p53- [7–10], FAS-mediated apoptosis and ras-mediated epidermal factor receptor-dependent angiogenesis [11–13]. ROS induce CKIp21 (Cip1), a gene causing permanent growth arrest/senescence and inactivate PTEN, a tumor suppressor protein causing cell death and senescence [14,15]. Degradation of hypoxia-inducible factor (HIF) that is strongly associated with angiogenesis is regulated by prolyl hydroxylases and asparagine hydroxylases also by a oxygen-dependent mechanism [16–18] (Fig. 2). Thus oxidants and cellular redox state are important determinants regulating cell growth, survival and expression of tumor suppressor genes.

2. Major antioxidant and thiol-disulfide regulating pathways in mammalian cells and human lung

Normal human lung is efficiently protected and "buffered" against exogenous free radicals. Besides classical antioxidant enzymes (AOEs), epithelial lining fluid contains small molecular weight antioxidants and proteins including a tripeptide glutathione (reduced glutathione, GSH) [19], mucin

* Corresponding author. Fax: +358-9-47176107.
E-mail address: vuokko.kinnula@helsinki.fi (V.L. Kinnula).

Abbreviations: AOE, antioxidant enzyme; CAT, catalase; $CYTP450_{ox}$, cytochrome P450 oxidase; CuZnSOD, copper–zinc superoxide dismutase; ECSOD, extracellular superoxide dismutase; G-6-P-D, glucose-6-phosphate dehydrogenase; γGCS, gamma glutamyl cysteinyl synthetase; GPX, glutathione peroxidase; GR, glutathione reductase; GRX, glutaredoxin; GS, glutathione synthase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-*S*-transferase; GT, glutamyl transpeptidase; H_2O_2, hydrogen peroxide; HIF, hypoxia-inducible factor; HOBr, hypobromide; HOCl, hypochloride; $OH^\cdot$, hydroxyl radical; MITO, mitochondria; MMP, matrix methalloproteinase; MnSOD, manganese superoxide dismutase; MRP, multidrug resistance protein; $NADPH_{ox}$, NADPH oxidase; NO, nitric oxide; $ONOO^-$, peroxynitrite; O_2^-, superoxide radical; PRX, peroxiredoxin; RNS, reactive nitrogen species; ROS, reactive oxygen species; RSS, reactive sulphur species; SOD, superoxide dismutase; TGFβ, transforming growth factor beta; TRX, thioredoxin; VEGF, vascular endothelial growth factor; XAO, xanthine oxidase

0014-5793/$22.00
doi:10.1016/j.febslet.2004.05.045

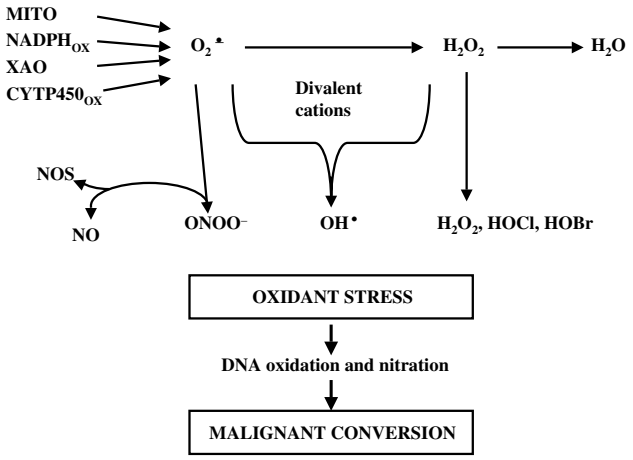

Fig. 1. Generation of ROS and RNS with consequent effects on malignant conversion. MITO, mitochondria; $NADPH_{ox}$, NADPH oxidase; XAO, xanthine oxidase; $CYTP450_{ox}$, cytochrome P450 oxidase; O_2^-, superoxide radical; H_2O_2, hydrogen peroxide; NO, nitric oxide; $ONOO^-$, peroxynitrite; $OH^\bullet$, hydroxyl radical; HOCl, hypochloride; HOBr, hypobromide.

glycoproteins [20], proteins capable in binding metals (transferrin, ferritin, ceruloplasmin, lactoferrin) and lipid (vitamin E) and water (vitamin C) soluble vitamins [22]. Furthermore, AOEs and related proteins have a highly compartmentalized localization being also expressed in all cell organelles (like mitochondria) and extracellularly.

Major human AOEs include superoxide dismutases (SODs), catalase (CAT), enzymes associated with GSH metabolism and its synthesis such as glutathione peroxidases (GPX) and glutamate cysteine ligase (gamma glutamyl cysteinyl synthetase, γGCS). There are three SODs, all of which are expressed also in human lung. Manganese SOD (MnSOD) is mitochondrial, CuZnSOD cytosolic and extracellular, ECSOD mainly extracellular [22–24]. The decomposition of H_2O_2 occurs by GSH-dependent enzymes and catalase which are located in the cytosolic compartment and peroxisomes (Fig. 3). Among these mechanisms GSH (glutamate–cysteine–glycine) is abundatly localized to the epithelial lining fluid of human lung [19] and its reaction pathways are tightly linked to the reactions of other thiol-containing proteins, that participate not only in scavenging of H_2O_2 but also to the regulation of the redox balance of the cells.

In addition to the classical AOEs, human lung expresses several thiol-containing proteins suggested to be major contributors to the redox environment of the cells. These proteins contain thiol-groups such as amino acid cysteine in their active centers including the families of thioredoxins (TRX1 and TRX2), thioredoxin reductases (TRR1 and TRR2), peroxiredoxins (PRXs I–VI) and glutaredoxins (GRXI, GRX2) [25,26]. Their cell specific expression in human lung is located mainly to alveolar macrophages, bronchial epithelial cells and alveolar epithelium, critical areas in the oxidant protection of human lung [27–30]. Besides these classical AOEs and the proteins of TRX, PRX and GRX families, other detoxification and oxidant-induced gene products may play a significant role in the primary defense of human lung. Glutathione-*S*-transferases (GSTs) belong to a group of detoxification enzymes that also require intracellular thiol tripeptide GSH for their catalytic activity as is also the case with the multidrug resistance proteins (MRPs) in the transport of various drugs from the cells. Both GSTs and MRPs have earlier been extensively investigated and reviewed [31–39]. Overall, human lung has a highly specialized antioxidative and redox-modulatory machinery that is expressed in the airways and functions as a major regulator of the cellular redox state both in non-malignant and malignant cells and their surroundings.

2.1. Superoxide dismutases and catalase in lung and pleural malignancies

Among the classical AOEs, MnSOD has been most widely investigated in malignant cells. In vitro studies and experi-

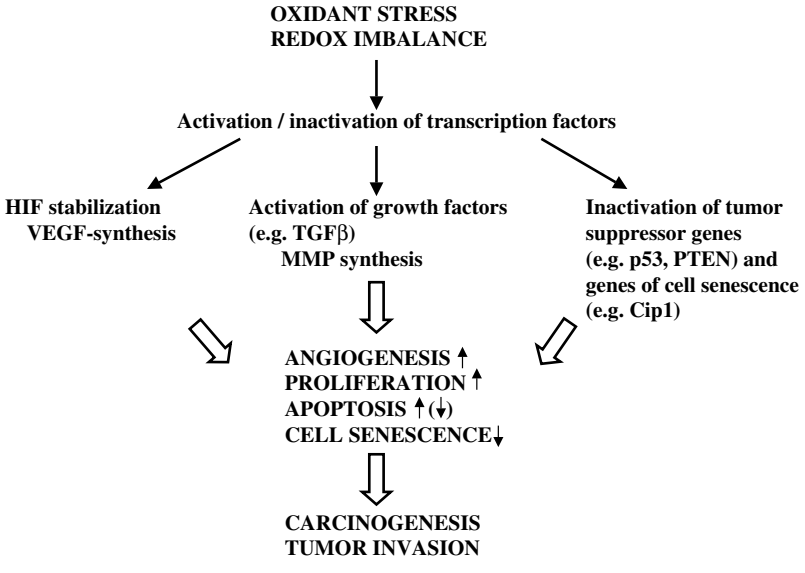

Fig. 2. Effects of oxidant stress on the stabilization of HIF with increased synthesis of vascular endothelial growth factor (VEGF), activation of growth factors such as transforming growth factor beta (TGFβ) and increased synthesis of matrix methalloproteinases (MMPs) and inactivation of tumor suppressor genes (e.g., p53 and PTEN).

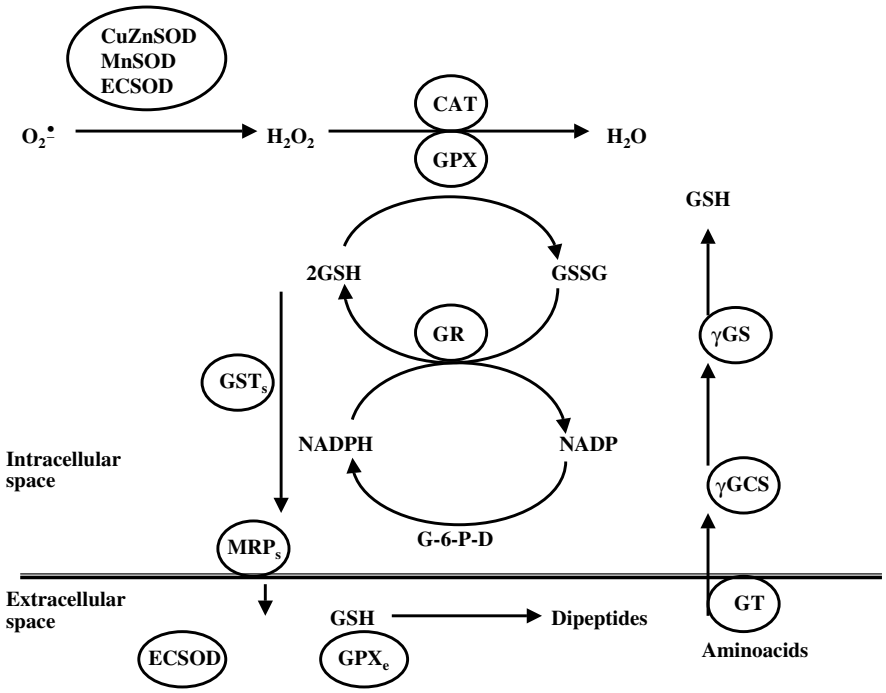

Fig. 3. Major antioxidant enzymes and related pathways in human tissues. CuZnSOD, copper–zinc superoxide dismutase; MnSOD, manganese SOD; ECSOD, extracellular SOD; CAT, catalase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-*S*-transferase; MRP, multidrug resistance protein; G-6-P-D, glucose-6-phosphate dehydrogensase; γGT, gamma glutamyl transpeptidase; γGCS, gamma glutamyl cysteinyl synthetase (glutamate cysteine ligase); GS, glutathione synthase; GPX_e, extracellular glutathione peroxidase.

mental models have concluded that overexpression of MnSOD by transfection decreases the growth, proliferation, and invasion of cultured malignant cells and may decrease tumor growth in experimental animal models in vivo [40–45]. Most these studies have introduced one enzyme such as MnSOD to the cells. This type of manipulation leads to disturbance of the natural oxidant/antioxidant balance of the cell, and therefore, does not reflect the situation in vivo. The results may thus be incorrectly interpreted if they are directly extrapolated to human studies The final and net effect of MnSOD is most likely related to the redox-milieu of the cells, which has been found to be related to the balance of MnSOD and some H_2O_2 scavenging enzymes in the cells [46].

MnSOD expression in normal lung and there in bronchial epithelium, alveolar epithelium and alveolar macrophages is low [47–49]. The promoter of MnSOD contains binding sites for a number of transcription factors reviewed by Zelko et al. [50], which explains the high inducibility of MnSOD both by cytokines, radiation and cytotoxic drugs [47,50–52] and its downregulation [53,54]. There is clear evidence that functional MnSOD polymorphism is associated with the development of lung cancer and possibly pleural mesothelioma, and also that MnSOD may be altered in precancerous tissues such as in dysplatic bronchial epithelium [55–58]. In contrast what one might expect from in vitro studies, several invasive and resistant tumors show high MnSOD levels or activities including brain tumors and various gastrointestinal malignancies [59–64]. However, no consistent results on the MnSOD expression in lung cancer have been reported [65–68], but a large study recently showed significantly elevated levels of MnSOD also in lung cancer [68]. More consistenly MnSOD (the mRNA, protein and/or activity) is highly elevated in malignant pleural mesothelioma, a tumor with poor prognosis and high resistance to chemotherapies and radiation [69–72]. Mesothelioma cells with highest (10-fold) MnSOD activity also possess elevated resistance to oxidants such as menadione and to cytotoxic drugs such as epirubicin [69], and cell lines expressing high MnSOD show decreased apoptosis [72]. Thus, malignant pleural mesotheliomas contain highly elevated MnSOD in the tumor cells with potential consequences to tumor behavior and drug resistance.

CuZnSOD is a cytosolic relatively constitutive enzyme being mainly expressed in bronchial epithelium [48]. A number of CuZnSOD functional polymorphisms have been characterized [57], but no associations have been shown between CuZnSOD polymorphisms and development of lung diseases. CuZnSOD contains several binding sites for transcription factors [73], but it is not induced by cytokines or ROS to the same extent as MnSOD in the lung [47,52] although it may be induced by radiation and xenochemicals [74]. There are some studies showing no dramatic changes of the messenger or protein levels of CuZnSOD in lung malignancies [67,68], while as with MnSOD, its overexpression has been reported in mesothelioma cells [75].

The expression of ECSOD in the lung is high [76,77], but very little is known about its significance in any malignancy. Functional polymorphic variant of ECSOD (that is rare, 3–6% in various populations) decreases the anchoring of ECSOD to negatively charged polysaccharides such as heparin in the extracellular matrix where this enzyme is mainly located [78]. There are, however, no studies on this polymorphism in malignant lung diseases. Recent study has shown that the ex-

pression of ECSOD is significantly lower in lung cancer than in the normal lung [79]. Since ECSOD is located in the extracellular space, its low expression in lung cancer may have fundamental importance in tumor invasion which is regulated by the cellular redox state.

Catalase represents a H_2O_2 scavenging enzyme with optimal activity at high H_2O_2 concentrations. In the lung, catalase is localized mainly in alveolar macrophages and alveolar epithelium [52]. This enzyme is relatively constitutive, no major induction has been reported by cytokines or oxidants in the lung. Catalase has not usually been connected to malignancies, but its expression in mesothelioma is high [80] suggesting that also this enzyme may be connected to highly resistant invasive tumors. This phenomenon may also have clinical significance since inhibition of catalase in vitro by aminotriaxole has shown to potentiate oxidant toxicity in mesothelioma cells [81].

2.2. Thiol modulating proteins and human lung and pleural malignancies

There is relatively good evidence that thiol related detoxification mechanisms such as GSTs and MRPs are closely associated with human lung malignancies and their resistance to cytotoxic drugs [34–39]. Relatively little is, however, known about the enzymes directly related to GSH synthesis and thiol-containing redox modulator proteins TRXs, PRXs or GRXs in malignant diseases. The rate limiting enzyme in GSH synthesis is glutamate cysteine ligase (i.e., γGCS). It consists of two subunits: a heavy subunit with catalytic function (γGCSh) and a light subunit with a regulatory role (γGCSl). Both subunits are expressed mainly in bronchial epithelium [82]. Especially the heavy subunit is induced by oxidants and oxidant generating anticancer drugs, such as cisplatin and doxorubicin, possibly by provoking oxidative stress in cancer cells [83]. In the only reported study on γGCS-mRNA expression in mesothelioma cell lines, also γGCSh-mRNA was co-ordinately overexpressed with MRP and the expression correlated with doxorubicin resistance [84]. Recent studies have also shown that malignat mesothelioma represents a tumor with high expression of γGCSh; moreover depletion of GSH by inhibiting γGCS both in mesothelioma and lung adenocarcinoma cells potentiated oxidant and drug-induced cell damage in vitro [81,85]. Overall there is strong evidence that GSH associated mechanisms and especially γGCSh are highly upregulated in malignant lung diseases and GSH depletion would be favourable for cell killing.

Thiol proteins TRX, TRR, PRX and GRX can be hypothesized to have fundamental role both in carcinogenesis and tumor progression. TRX is a 12 kDa multifunctional protein catalyzing protein disulfide reductions in conjuction with TRR. Besides being a powerful regulator of cellular redox state, TRX increases cell proliferation and resistance of various cells to oxidants and drugs [25,86]. The TRX system is also induced at least by oxidant stress, tumor promoting agents and cytotoxic drugs [26,50]. In agreement with these notions, TRX mRNA was prominently expressed in mesothelioma cells [75] and immunohistochemical studies confirmed high expression of TRX and/or TRR proteins both in malignant mesothelioma and lung cancer [71,87]. Given the important role of this system in the drug resistance, TRX may also increase the resistance of lung carcinoma and mesothelioma to the cytotoxic drugs and radiation.

Peroxiredoxins constitute another thiol-containing antioxidant family of proteins that have been recently detected from human lung, and there mainly in bronchial epithelium, alveolar epithelium and alveolar macrophages [27]. PRXs are closely associated with TRX-dependent reactions, being also called as thioredoxin peroxidases. Normal healthy lung appears to express abundantly of nearly all these proteins. These proteins are also stress-inducible being associated with cell signalling pathways; they also participate in the cellular antioxidant defense, at least part of them induce cell proliferation and protect cells from undergoing apoptosis [88–90]. Most proteins of the PRX family (PRX I, II, III, V, VI) are highly expressed in malignant mesothelioma [29] and some of them also in lung cancer [91]. GRX family of proteins is linked more to GSH pathways, their role in lung malignancies is unknown. It appears that PRXs represent a new family of redox modulatory thiol proteins with potential consequences on cell proliferation, tumor growth and resistance.

3. Conclusions

It is known that lung antioxidant levels are decreased in non-malignant lung disorders such as in asthma and chronic obstructive lung disease [47,82], whereas the primary antioxidant mechanisms are high, probably induced in lung malignancies, and especially in malignant mesothelioma. In this disease high antioxidant capacity of the tumor cells can be hypothesized to lead to the observed high resistance to cytotoxic drugs and radiation. Even more importantly recently characterized families of thiol proteins may prove to have fundamental role in cancer biology, cellular redox state being one of the major modulators of a number of growth factors associated with angiogenesis and cell proliferation. These thiol proteins such as TRXs and PRXs not only increase cell survival and proliferation but also protect both non-malignant and malignant cells against oxidants, radiation and chemotherapies. However, the functional significance of these thiol proteins is still unresolved and waits future studies.

Acknowledgements: This study was partly supported by the Cancer Society of Finland, Juselius Foundation and Finnish Antituberculosis Association Foundation.

References

[1] Ames, B.N. (1983) Science 221, 1256–1264.
[2] Church, D.F. and Pryor, W.A. (1985) Environ. Health Perspect. 64, 111–126.
[3] Osada, H. and Takahashi, T. (2002) Oncogene 21, 7421–7434.
[4] Cugell, D.W. and Kamp, D.W. (2004) Chest 125, 1103–1117.
[5] Burdon, R.H. (1995) Free Radic. Biol. Med. 18, 775–794.
[6] Sun, Y. and Oberley, L.W. (1996) Free Radic. Biol. Med. 21, 335–348.
[7] Brunelle, J.K. and Chandel, N.S. (2002) Apoptosis 7, 475–482.
[8] Chandel, N.S., Vander Heiden, M.G., Thompson, C.B. and Schumacker, P.T. (2000) Oncogene 19, 3840–3848.
[9] Meplan, C., Richard, M.J. and Hainaut, P. (2000) Biochem. Pharmacol. 59, 25–33.
[10] Vafa, O., Wade, M., Kern, S., Beeche, M., Pandita, T.K., Hampton, G.M. and Wahl, G.M. (2002) Mol. Cell 9, 1031–1044.
[11] Casanova, M.L., Larcher, F., Casanova, B., Murillas, R., Fernandez-Acenero, M.J., Villanueva, C., Martinez-Palacio, J., Ullrich, A., Conti, C.J. and Jorcano, J.L. (2002) Cancer Res. 62, 3402–3407.

[12] Kasahara, Y., Iwai, K., Yachie, A., Ohta, K., Konno, A., Seki, H., Miyawaki, T. and Taniguchi, N. (1997) Blood 89, 1748–1753.
[13] Suhara, T., Fukuo, K., Sugimoto, T., Morimoto, S., Nakahashi, T., Hata, S., Shimizu, M. and Ogihara, T. (1998) J. Immunol. 160, 4042–4047.
[14] Lee, S.R., Yang, K.S., Kwon, J., Lee, C., Jeong, W. and Rhee, S.G. (2002) J. Biol. Chem. 277, 20336–20342.
[15] Macip, S., Igarashi, M., Fang, L., Chen, A., Pan, Z.Q., Lee, S.W. and Aaronson, S.A. (2002) EMBO J. 21, 2180–2188.
[16] Jaakkola, P., Mole, D.R., Tian, Y.M., Wilson, M.I., Gielbert, J., Gaskell, S.J., Kriegsheim, A., Hebestreit, H.F., Mukherji, M., Schofield, C.J., Maxwell, P.H., Pugh, C.W. and Ratcliffe, P.J. (2001) Science 292, 468–472.
[17] Welsh, S.J., Bellamy, W.T., Briehl, M.M. and Powis, G. (2002) Cancer Res. 62, 5089–5095.
[18] Lando, D., Peet, D.J., Whelan, D.A., Gorman, J.J. and Whitelaw, M.L. (2002) Science 295, 858–861.
[19] Cantin, A.M., North, S.L., Hubbard, R.C. and Crystal, R.G. (1987) J. Appl. Physiol. 63, 152–157.
[20] Adler, K.B. and Li, Y. (2001) Am. J. Respir. Cell Mol. Biol. 25, 397–400.
[22] Halliwell, B. and Gutteridge, J. (1996) Free Radicals in Biology and Medicine. Oxford Claredon Press.
[22] Slot, J.W., Geuze, H.J., Freeman, B.A. and Crapo, J.D. (1986) Lab Invest. 55, 363–371.
[23] Weisiger, R.A. and Fridovich, I. (1973) J. Biol. Chem. 248, 4793–4796.
[24] Oury, T.D., Chang, L.Y., Marklund, S.L., Day, B.J. and Crapo, J.D. (1994) Lab Invest. 70, 889–898.
[25] Holmgren, A. (2000) Antioxid. Redox. Signal. 2, 811–820.
[26] Powis, G. and Montfort, W.R. (2001) Annu. Rev. Pharmacol. Toxicol. 41, 261–295.
[27] Kinnula, V.L., Lehtonen, S., Kaarteenaho-Wiik, R., Lakari, E., Paakko, P., Kang, S.W., Rhee, S.G. and Soini, Y. (2002) Thorax 57, 157–164.
[28] Peltoniemi, M., Kaarteenaho-Wiik, R., Säily, M., Sormunen, R., Pääkkö, P., Holmgren, A., Soini, Y. and Kinnula, V.L. (2004) Human Pathol., in press.
[29] Kinnula, V.L., Lehtonen, S., Sormunen, R., Kaarteenaho-Wiik, R., Kang, S.W., Rhee, S.G. and Soini, Y. (2002) J. Pathol. 196, 316–323.
[30] Tiitto, L., Kaarteenaho-Wiik, R., Sormunen, R., Holmgren, A., Paakko, P., Soini, Y. and Kinnula, V.L. (2003) J. Pathol. 201, 363–370.
[31] Keppler, D. (1999) Free Radic. Biol. Med. 27, 985–991.
[32] Borst, P., Evers, R., Kool, M. and Wijnholds, J. (2000) J. Natl. Cancer Inst. 92, 1295–1302.
[33] Rappa, G., Lorico, A., Flavell, R.A. and Sartorelli, A.C. (1997) Cancer Res. 57, 5232–5237.
[34] Zhang, K., Mack, P. and Wong, K.P. (1998) Int. J. Oncol. 12, 871–882.
[35] Ishikawa, T. (1992) Trends Biochem. Sci. 17, 463–468.
[36] Lai, S.L., Goldstein, L.J., Gottesman, M.M., Pastan, I., Tsai, C.M., Johnson, B.E., Mulshine, J.L., Ihde, D.C., Kayser, K. and Gazdar, A.F. (1989) J. Natl. Cancer Inst. 81, 1144–1150.
[37] Campling, B.G., Young, L.C., Baer, K.A., Lam, Y.M., Deeley, R.G., Cole, S.P. and Gerlach, J.H. (1997) Clin. Cancer Res. 3, 115–122.
[38] Soini, Y., Jarvinen, K., Kaarteenaho-Wiik, R. and Kinnula, V. (2001) Ann. Oncol. 12, 1239–1245.
[39] Young, L.C., Campling, B.G., Voskoglou-Nomikos, T., Cole, S.P., Deeley, R.G. and Gerlach, J.H. (1999) Clin. Cancer Res. 5, 673–680.
[40] Bravard, A., Sabatier, L., Hoffschir, F., Ricoul, M., Luccioni, C. and Dutrillaux, B. (1992) Int. J Cancer 51, 476–480.
[41] Oberley, L.W. and Oberley, T.D. (1997) Role of antioxidant entzymes in the cancer phenotype. in: Oxygen, Gene Expression and Cellular Function (Clerch, L.B. and Massaro, D.J., Eds.), pp. 279–308, Marcel Dekker, Washington, DC.
[42] Yan, T., Oberley, L.W., Zhong, W. and St Clair, D.K. (1996) Cancer Res. 56, 2864–2871.
[43] Li, J.J., Oberley, L.W., St Clair, D.K., Ridnour, L.A. and Oberley, T.D. (1995) Oncogene 10, 1989–2000.
[44] St Clair, D.K., Wan, X.S., Oberley, T.D., Muse, K.E. and St Clair, W.H. (1992) Mol. Carcinog. 6, 238–242.
[45] Zhong, W., Oberley, L.W., Oberley, T.D. and St Clair, D.K. (1997) Oncogene 14, 481–490.
[46] Kinnula, V.L. and Crapo, J.D. (2004) Free Radic. Biol. Med. 36, 718–744.
[47] Kinnula, V.L. and Crapo, J.D. (2003) Am. J. Respir. Crit Care Med. 167, 1600–1619.
[48] Lakari, E., Paakko, P. and Kinnula, V.L. (1998) Am. J. Respir. Crit. Care Med. 158, 589–596.
[49] Lakari, E., Paakko, P., Pietarinen-Runtti, P. and Kinnula, V.L. (2000) Am. J. Respir. Crit. Care Med. 161, 615–621.
[50] Zelko, I.N., Mariani, T.J. and Folz, R.J. (2002) Free Radic. Biol. Med. 33, 337–349.
[51] Janssen, Y.M., Van Houten, B., Borm, P.J. and Mossman, B.T. (1993) Lab Invest. 69, 261–274.
[52] Kinnula, V.L., Crapo, J.D. and Raivio, K.O. (1995) Lab Invest. 73, 3–19.
[53] Pani, G., Bedogni, B., Anzevino, R., Colavitti, R., Palazzotti, B., Borrello, S. and Galeotti, T. (2000) Cancer Res. 60, 4654–4660.
[54] Jackson, R.M., Parish, G. and Ho, Y.S. (1996) Am. J. Physiol. 271, L955–L962.
[55] Wang, L.I., Miller, D.P., Sai, Y., Liu, G., Su, L., Wain, J.C., Lynch, T.J. and Christiani, D.C. (2001) J. Natl. Cancer Inst. 93, 1818–1821.
[56] Hirvonen, A., Tuimala, J., Ollikainen, T., Linnainmaa, K. and Kinnula, V. (2002) Cancer Lett. 178, 71–74.
[57] Forsberg, L., de Faire, U. and Morgenstern, R. (2001) Arch. Biochem. Biophys. 389, 84–93.
[58] Soini, Y., Kaarteenaho-Wiik, R., Paakko, P. and Kinnula, V. (2003) Lung Cancer 39, 15–22.
[59] Cobbs, C.S., Levi, D.S., Aldape, K. and Israel, M.A. (1996) Cancer Res. 56, 3192–3195.
[60] Haapasalo, H., Kylaniemi, M., Paunul, N., Kinnula, V.L. and Soini, Y. (2003) Brain Pathol. 13, 155–164.
[61] Landriscina, M., Remiddi, F., Ria, F., Palazzotti, B., De Leo, M.E., Iacoangeli, M., Rosselli, R., Scerrati, M. and Galeotti, T. (1996) Br. J. Cancer 74, 1877–1885.
[62] Janssen, A.M., Bosman, C.B., Sier, C.F., Griffioen, G., Kubben, F.J., Lamers, C.B., van Krieken, J.H., van de Velde, C.J. and Verspaget, H.W. (1998) Br. J. Cancer 78, 1051–1057.
[63] Kawamura, N., Suzuki, K., Ishikawa, M., Iizuka, S., Miyake, M., Mino, M. and Taniguchi, N. (1992) Free Radic. Biol. Med. 12, 281–286.
[64] Toh, Y., Kuninaka, S., Oshiro, T., Ikeda, Y., Nakashima, H., Baba, H., Kohnoe, S., Okamura, T., Mori, M. and Sugimachi, K. (2000) Int. J. Oncol. 17, 107–112.
[65] Jaruga, P., Zastawny, T.H., Skokowski, J., Dizdaroglu, M. and Olinski, R. (1994) FEBS Lett. 341, 59–64.
[66] Guner, G., Islekel, H., Oto, O., Hazan, E. and Acikel, U. (1996) Cancer Lett. 103, 233–239.
[67] Coursin, D.B., Cihla, H.P., Sempf, J., Oberley, T.D. and Oberley, L.W. (1996) Histol. Histopathol. 11, 851–860.
[68] Chung-man, H.J., Zheng, S., Comhair, S.A., Farver, C. and Erzurum, S.C. (2001) Cancer Res. 61, 8578–8585.
[69] Kinnula, V.L., Pietarinen-Runtti, P., Raivio, K., Kahlos, K., Pelin, K., Mattson, K. and Linnainmaa, K. (1996) Free Radic. Biol. Med. 21, 527–532.
[70] Kahlos, K., Anttila, S., Asikainen, T., Kinnula, K., Raivio, K.O., Mattson, K., Linnainmaa, K. and Kinnula, V.L. (1998) Am. J. Respir. Cell Mol. Biol. 18, 570–580.
[71] Kahlos, K., Soini, Y., Paakko, P., Saily, M., Linnainmaa, K. and Kinnula, V.L. (2000) Int. J. Cancer 88, 37–43.
[72] Kahlos, K., Paakko, P., Kurttila, E., Soini, Y. and Kinnula, V.L. (2000) Br. J. Cancer 82, 1022–1029.
[73] Kim, H.T., Kim, Y.H., Nam, J.W., Lee, H.J., Rho, H.M. and Jung, G. (1994) Biochem. Biophys. Res. Commun. 201, 1526–1533.
[74] Inoue, N., Ramasamy, S., Fukai, T., Nerem, R.M. and Harrison, D.G. (1996) Circ. Res. 79, 32–37.
[75] Rihn, B.H., Mohr, S., McDowell, S.A., Binet, S., Loubinoux, J., Galateau, F., Keith, G. and Leikauf, G.D. (2000) FEBS Lett. 480, 95–100.
[76] Marklund, S.L. (1984) Biochem. J. 222, 649–655.
[77] Oury, T.D., Day, B.J. and Crapo, J.D. (1996) Lab Invest. 75, 617–636.

[78] Sandstrom, J., Nilsson, P., Karlsson, K. and Marklund, S.L. (1994) J. Biol. Chem. 269, 19163–19166.
[79] Svensk, A.-M., Soini, Y., Pääkkö, P., Hirvikoski, P. and Kinnula, V.L. (2004) Am. J. Clin. Pathol., in press.
[80] Kahlos, K., Soini, Y., Sormunen, R., Kaarteenaho-Wiik, R., Paakko, P., Linnainmaa, K. and Kinnula, V.L. (2001) Cancer 91, 1349–1357.
[81] Kinnula, K., Linnainmaa, K., Raivio, K.O. and Kinnula, V.L. (1998) Br. J. Cancer 77, 1097–1102.
[82] Harju, T., Kaarteenaho-Wiik, R., Soini, Y., Sormunen, R. and Kinnula, V.L. (2002) Am. J. Respir. Crit. Care Med. 166, 754–759.
[83] Galloway, D.C., Blake, D.G., Shepherd, A.G. and McLellan, L.I. (1997) Biochem. J. 328 (Pt 1), 99–104.
[84] Tipnis, S.R., Blake, D.G., Shepherd, A.G. and McLellan, L.I. (1999) Biochem. J. 337 (Pt 3), 559–566.
[85] Jarvinen, K., Soini, Y., Kahlos, K. and Kinnula, V.L. (2002) Hum. Pathol. 33, 748–755.
[86] Arner, E.S. and Holmgren, A. (2000) Eur. J. Biochem. 267, 6102–6109.
[87] Soini, Y., Kahlos, K., Napankangas, U., Kaarteenaho-Wiik, R., Saily, M., Koistinen, P., Paakko, P., Holmgren, A. and Kinnula, V.L. (2001) Clin. Cancer Res. 7, 1750–1757.
[88] Kang, S.W., Chae, H.Z., Seo, M.S., Kim, K., Baines, I.C. and Rhee, S.G. (1998) J. Biol. Chem. 273, 6297–6302.
[89] Kim, H., Lee, T.H., Park, E.S., Suh, J.M., Park, S.J., Chung, H.K., Kwon, O.Y., Kim, Y.K., Ro, H.K. and Shong, M. (2000) J. Biol. Chem. 275, 18266–18270.
[90] Zhou, Y., Kok, K.H., Chun, A.C., Wong, C.M., Wu, H.W., Lin, M.C., Fung, P.C., Kung, H. and Jin, D.Y. (2000) Biochem. Biophys. Res. Commun. 268, 921–927.
[91] Lehtonen, S.T., Svensk, A.-M., Soini, Y., Pääkkö, P., Hirvikoski, P., Kang, S.W., Säily, M. and Kinnula, V.L. (2004) Int. J. Cancer, in press.