for C57BL/6J mice PCPMS equal 1.85 and these animals almost have not spontaneous tumors. Moreover, lifespan of CBA mice is significantly lower than lifespan of C57BL/6J mice. We are suggesting that PCPMS will be helpful for early prognosis of diseases, preventive treatment and enzyme corrections as well as for individualization of chemotherapy.

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## *IN VIVO* ESR STUDY ON RADICAL GENERATION IN MOUSE SKIN UNDER ULTRAVIOLET LIGHT

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There have been little reports about *in vivo* evidence of free radical generation during exposure to ultraviolet light (UV), although free radicals may be involved in the injuries caused by UV irradiation. In this study, the induction of radical reaction was examined in skin of living mouse under UV light by using in vivo ESR spectroscopy with a nitroxyl radical, 3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-N-oxyl (carbamoyl-PROXYL), as a redox probe. An aqueous solution of carbamoyl-PROXYL was injected intravenously to an anesthetized mouse, and L-band ESR spectra of the probe were recorded at the dorsal region of either hair-removed ddY mice or hairless mice. A surface-coil-type resonator was used to detect carbamoyl-PROXYL in skin. ESR signal of carbamoyl-PROXYL increased up to a few minutes after the injection and then decreased. The rate of the signal decay increased under irradiation with UV light. The increase was statistically significant. The increase of signal decay rate was not observed in mouse whose skin was removed and then returned, indicating that the enhancement of signal decay should occur in skin. The increase of signal decay rate was suppressed by pre-administration of a spin trapping reagent, N-t-butyl-α-phenylnitrone (PBN), while PBN did not change the decay rate for non-irradiated mouse. These observations suggest that the measurement of radical generation under UV light is possible by using in vivo ESR spectroscopy and a nitroxyl redox probe.

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## OXIDIZED EICOSANOIDS IN UV-IRRADIATED HUMAN SKIN AND HaCaT-CULTURES AFTER ADMINISTRATION OF ANTI-INFLAMMATORY DRUGS USING MICRODIALYSIS TECHNIQUE

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UV-irradiation of the human skin leads to the induction of oxidative stress and inflammation mediated by reactive oxygen radicals, lipid peroxidation, liberation of arachidonic acid from membrane phospholipids and formation of prostaglandins and leucotrienes. Therefore, we investigated "lipid mediators", such as 8-iso-PGF2α, HETEs and LTB4 in the dermal interstitial fluid obtained in vivo by cutaneous microdialysis technique and in vitro from keratinocyte (HaCaT) cultures after UV-irradiation and application of diclofenac, a nonsteroidal anti-inflammatory drug. Defined areas on the volar forearm of 10 healthy volunteers were exposed to UVB irradiation (20-60 mJ/cm$^2$). After 3 or 24 hours, microdialysis membranes were cutaneously inserted beneath the irradiated area and diclofenac was administered topically. The membranes were perfused with isotonic saline solution and microdialysate samples were collected at 20 min intervals over up to 4 hours. Analysis of oxidized arachidonic acid derivatives using sensitive NICI-GC-MS showed enhanced amounts of 5-, 8-12- and 15-HETE, LTB4 and 8-iso-PGF2α after UV irradiation, which were suppressed to a different extent by topical application of diclofenac. Further investigations may show whether these new findings may also be relevant to validate therapeutical strategies for other inflammatory skin diseases.

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## OXIDATIVE DAMAGE AND MITOCHONDRIAL DYSFUNCTION IN THE PROGRESSION OF HUMAN COLORECTAL CANCER

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Colorectal cancer is one of the most frequent invasive tumours representing about 15% of all cancers in western countries. Tumour cells may produce large amounts of peroxides and this may contribute to their ability to mutate and damage normal tissues, and thereby facilitate tumour growth and invasion. The superoxide radical has been shown to be a potent promoter of mutagenesis in the colonic mucosa. Many cancer cells show low levels of antioxidant enzymes and only a few types, as colorectal cancer, have been found to exhibit elevated levels of superoxide dismutase activity (SOD). We assayed lipid peroxidation, and both citosolic and mitochondrial superoxide dismutase activities, in tumour and adjacent non-tumoral tissue of human colorectal carcinomas (CRC) in initial (IS) and advanced stages (AS). We found higher lipid peroxidation in the tumour than in adjacent non-tumoral tissue, in spite of a parallel increase in total SOD activity. When IS and AS were compared, CRC progression exhibited a significant decrease of Cu,Zn-SOD activity, whereas mitochondrial Mn-SOD activity remained unchanged. We determined NADH-cytochrome c reductase, succinate-cytochrome c reductase and cytochrome oxidase activities in isolated mitochondria from tumoral and non-tumoral tissues. NADH-cytochrome c reductase activity was significantly lower in tumour than non-tumour tissue, and in AS than IS. The results could be explained by the increased lipoperoxidation, and hence altered mitochondrial electron transfer was expected. Our results support the hypothesis that an increased SOD activity

in these cells does not protect against oxidative stress and sustained index of oxidative lesions could be involved in the chronic mutagenic pressure needed for clonal progression of human colorectal cancer cells.

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## CROSS-TALK OF NO AND OXYRADICALS CONSTITUTES A SUPERSYSTEM FOR THE REGULATION OF MORPHGENESIS AND THE SURVIVAL OF AEROBIC LIFE

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We previously showed that NO rapidly interacts with superoxide and molecular oxygen, thereby constituting a supersystem that regulates the circulatory status and mitochondrial electron transport particularly under physiologically low oxygen tensions. The cross-talk of NO and related oxyradicals also regulates ATP synthesis of a wide variety of bacteria, thus functioning as an essential defense system against pathogens. We recently found that this system also underlies the mechanism that triggers apoptosis of particular cells, thereby commanding morphgenesis and metamorphosis of various organisms, such as developing chick embryo, tadpoles, beetles and silk warms. Biochemical analysis revealed that functional modulation of mitochondria, such as fatty acid oxidation and membrane permeability transition by the supersystem play central roles in the regulation of bioenergetics and processes of embryonic development and metamorphosis of aerobic organisms. The critical roles of the supersystem driven by NO and oxyradicals in the survival, aging and malignant transformation of cells will be discussed.

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## TARGET GENES IN FENTON REACTION-INDUCED CARCINOGENISIS : A NOVEL CONCEPT

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Oxidative stress has been associated with carcinogenesis. In 1982, our laboratory established a fenton reaction-based carcinogenesis model in rodent kidney by the use of an iron chelate, ferric nitrilotriacetate (Fe-NTA). This model is unique in the following three aspects: 1)exclusively carcinoma, no sarcoma, is induced, 2)carcinomas induced are of highly malignant potential, and 3) a demonstration of increase in a variety of covalently modified molecules including 8-hydroxy-2'-deoxyguanosine, 4-hydroxy-2-nonenal (HNE) and HNE-modified proteins after administration of Fe-NTA. Since free radical reactions have been believed to show little preference for specific molecules especially *in vitro*, we undertook to answer the question whether "there is any specific target gene(s) in this carcinogenesis model". By analyzing $F_1$ hybrid rats, we identified that $p15^{INK4B}$/$p16^{INK4A}$ tumor suppressor genes are one of the major pathways responsible for this oxidative stress-induced carcinogenesis. Furthermore, by the use of fluorescent in situ hybridization at single –cell resolution, we found that the fraction of renal tubular cells with aneuploidy (1- or 3-signal) at the $p16^{INK4A}$ locus was significantly and specifically increased after repeated administration of Fe-NTA after 1 week. Thus, the $p16^{INK4A}$ locus is vulnerable to oxidative damage, leading to its allelic loss in weeks presumably due to the deficiency in replicating both of the alleles. This method is also useful for the screening of cancer chemopreventive agents.