Cancer and Excess Iron

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Keywords
Iron · Asbestos · Mesothelioma · Oxygen · Reactive oxygen species (ROS)

19.1 Case Report

A 62-year-old male presented transient loss of consciousness during dialysis and was transferred to the emergency room. Chest X-ray and computed tomography revealed prominent right pleural effusion with large tumor mass surrounding the contour of the right lung. The patient had been involved in demolition work of old buildings and water pipes for more than 25 years, where he was often exposed to asbestos fibers in the air. The patient suffered from diabetes mellitus for 18 years and started dialysis three times a week since 7 years ago due to diabetic nephropathy.

The patient was diagnosed as probable malignant mesothelioma at an advanced stage, based on needle biopsy of the lung tumor, and thereafter received chemotherapy. However, the chemotherapy was not very effective, and the patient died 3 months later due to pulmonary and renal failure. An autopsy was performed.

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19.2 Diagnosis

In general, diagnosis of cancer is based on histological examination of formalin-fixed paraffin-embedded sections under a microscope. Cancer is a word, representing malignant neoplasm, and is classified according to the differentiation of the cancer cells. If the cancer cells are differentiated toward glands, the cancer is called "adenocarcinoma," and if the cancer cells are differentiated toward stratified squamous cells, such as epidermis, the cancer is called "squamous cell carcinoma." Here the cancer cells were differentiated into mesothelial cells, so this cancer is called as "malignant mesothelioma." Mesothelial cells are flat one-layer lining cells of somatic cavities. The humans have three somatic cavities in the body, namely, pleural cavity, peritoneal cavity, and pericardial cavity, in which the lung, abdominal organs (stomach, small and large intestine, spleen, liver, etc.), and heart are accommodated, respectively. One of the major functions of mesothelial cells is to lubricate the somatic cavities for the movement of organs inside by secreting hyaluronic acid.

Autopsy examination revealed that the tumor was located at the periphery of the lung, thus consistent with malignant mesothelioma (Fig. 19.1). The histology of the tumor showed proliferation of atypical polygonal cells with a sheet or glandular structure (Fig. 19.2). Immunohistochemical analyses by monoclonal antibodies of these

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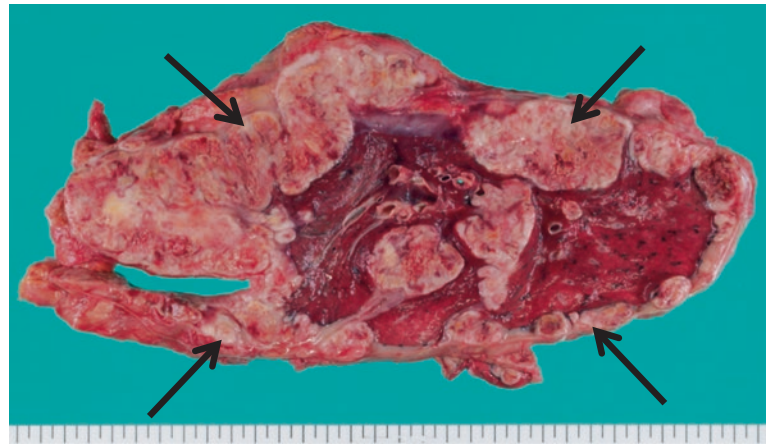

Fig. 19.1 Macroscopic appearance of pleural malignant mesothelioma in a human autopsied case. Note that whitish mass (indicated by arrows) surrounding the lung tissue is the invading malignant mesothelioma

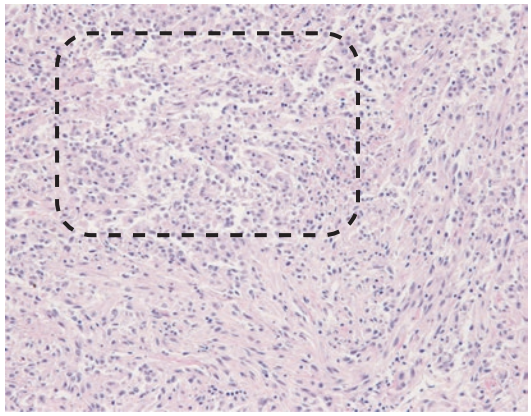

Fig. 19.2 Microscopic appearance of pleural malignant mesothelioma. Atypical mesothelial cells are proliferating with a sheet or glandular (areas surrounded by dotted line) structure (hematoxylin and eosin staining)

Table 19.1 Classification of carcinogenic agents

Habits	Smoking, alcohol
Living environments	UV, radiation, arsenic, PM2.5
Work environments	Asbestos, benzene, dichloromethane
Diet	High fat, low fiber
Infection	HTLV-1, hepatitis C virus, papilloma virus, helicobacter pylori
Chronic inflammation	Autoimmune diseases (Hashimoto thyroiditis, Crohn's disease, ulcerative colitis)
Hereditary (genetic)	Allelic loss/inactivation of tumor suppressor genes (*p53, VHL, MUTHY, BRCA*)

The items and carcinogenic agents are representative. *UV* ultraviolet, *PM* particulate matter, *HTLV* human T-cell leukemia virus

tumor cells were positive for cytokeratin (clones, AE1/3 and CAM5.2), calretinin, and podoplanin (clone D2–40) and negative for TTF-1 (a molecular marker of adenocarcinoma of lung origin). These results were consistent with mesothelial differentiation, and the diagnosis of malignant mesothelioma was established.

19.3 Biochemical and Molecular Perspectives

Cancer is one of the top causes of death in virtually all of the developed countries (Siegel et al. 2015) after the conquest of major infectious diseases, such as tuberculosis (Heesterbeek et al. 2015). Cancer patients are increasing now partially due to prolonged average lifetime (80.5 years for males and 86.8 years for females in Japan in 2016). Table 19.1 summarizes the classification of carcinogenic agents, most of which are associated with oxidative stress.

Oxidative stress is an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage. We cannot live without oxygen even for 5 min, but we do not obtain energy from oxygen. We obtain energy through diet. Oxygen works as a medium for electron flow. We use the characteristics of oxygen to be reduced four times independently to

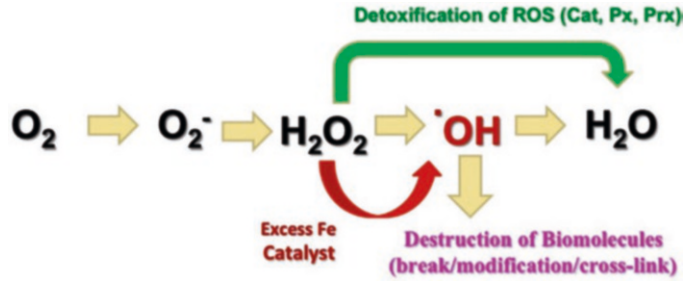

Fig. 19.3 Role of oxygen in the biological system. *Cat* catalase, *Px* peroxidase, *Prx* peroxiredoxin, *ROS* reactive oxygen species

Table 19.2 Association of excess iron with carcinogenesis in humans

Hereditary hemochromatosis	Liver	Hepatocellular carcinoma
Viral hepatitis B/C	Liver	Hepatocellular carcinoma
Ovarian endometriosis	Ovary	Adenocarcinoma
Exposure to asbestos	Somatic cavity (mesothelial cell), lung	Malignant mesothelioma, lung cancer

water (Fig. 19.3). During these reactions, reactive oxygen species (ROS) are generated, which account for a few % of oxygen consumed (Toyokuni 1999). Here superoxide (O_2^-) and hydrogen peroxide (H_2O_2) themselves are not so reactive but are used as signaling molecules in the cell. However, hydroxyl radical (·OH) is the most reactive chemical species in the biological system and damages all sorts of biomolecules nearby. If ·OH attacks DNA, several different kinds of DNA damages, strand breaks, base modifications, etc. are induced, which may lead to mutations (alteration of genome information). Generally, five or six cumulative mutations at oncogene or tumor suppressor genes in a single cell are necessary for carcinogenesis (Weinberg 2013). Importantly, a generation of ·OH is a chemical reaction catalyzed by Fe(II) and is called Fenton reaction: $Fe(II) + H_2O_2 \rightarrow Fe(III) + {\cdot}OH + OH^-$ (Toyokuni 1996).

Iron is the most abundant transition metal in our body, and adult male humans contain approximately 4 grams of iron. Sixty percentage of them are present in red blood cells as heme in hemoglobin. Usually catalytic or free iron scarcely exists in or outside of the cell except for lysosomes, because of efficient clearing systems such as serum transferrin (iron transport) and cytosolic ferritin (iron storage) proteins. However, excess iron produces catalytic iron in vivo and is significantly associated with carcinogenesis (Toyokuni 2009b). Table 19.2 summarizes excess iron-associated carcinogenesis in humans, supported by epidemiological data (Toyokuni 2016).

Asbestos is a natural fibrous mineral, found in asbestos mines (Fig. 19.4). Because it is a stone, it is resistant to heat, acid, and friction, so it has been used in a huge amount worldwide in the last century. It is a fiber of micrometer-order length and several hundred nanometer-order diameter, so it can easily fly in the air and miners and workers are exposed at the respiratory tract (IARC 2012; Oury et al. 2014). Asbestos was used even for the wall surface of houses in the 1970s to be preventive against fire, and the general population was also exposed. Epidemiologists noticed that asbestos is responsible for malignant mesothelioma in the 1970s, but it took for a while to ban asbestos legally. For example, Japan banned all kinds of asbestos in 2006 and Canada in 2012. There are two different types of asbestos: serpentine (chrysotile, white asbestos) and amphibole (crocidolite, blue asbestos; amosite, brown asbestos). Amphiboles show more rigidity, and ~30% of its content is iron (IARC 2012; Oury et al. 2014). However, it has become clear that white

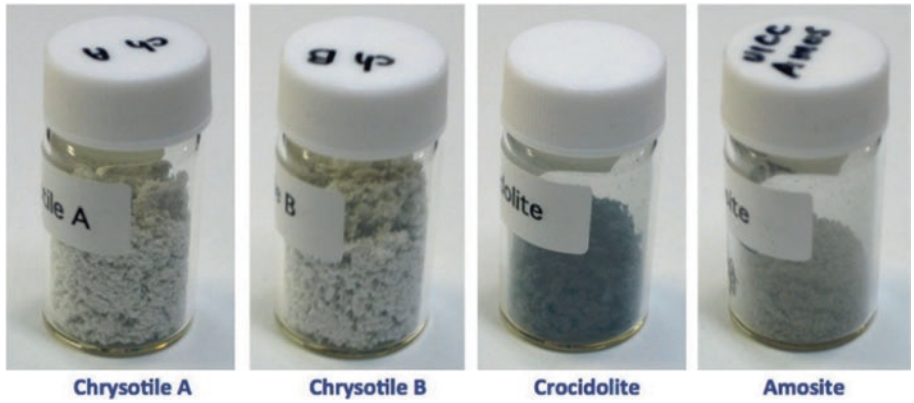

Fig. 19.4 Standard asbestos fibers distributed for experiments by UICC (Union for International Cancer Control)

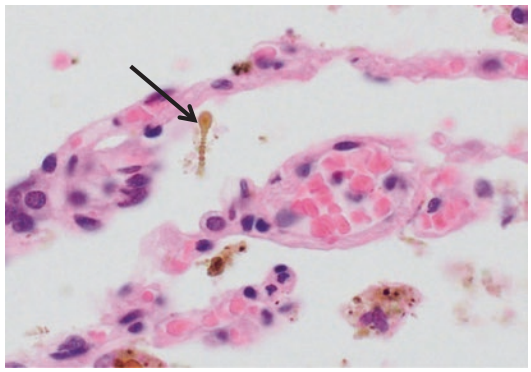

Fig. 19.5 Asbestos body (arrow) formed in a human lung (hematoxylin and eosin staining). Note the dumbbell-like appearance and golden brown color of asbestos body, which accumulated iron as hemosiderin

asbestos (chrysotile) which contains no iron in its content is also potently carcinogenic (Jiang et al. 2012). It is well known that there is a long incubation period of 30–40 years for the induction of malignant mesothelioma after asbestos exposure (IARC 2012; Oury et al. 2014).

Now we know what is happening, based on the data of animal experiments. Very long incubation period is the time required for the fibers to pass through the lung tissue into the pleural cavity. Because asbestos is foreign to our body, alveolar macrophages engulf asbestos fibers as soon as they find the fibers and try to digest them or to carry them to regional lymph nodes. However, neither of them is possible for the macrophages, and they die after engulfing asbestos fibers because they are sharp and long enough to break the plasma membrane of macrophages. Due to the negative pressure of the pleural cavity, the asbestos fibers go peripherally over years as they kill the macrophages and other cells (Toyokuni 2009a; Chew and Toyokuni 2015).

It is now known that all the asbestos fibers have affinity to hemoglobin and histones (Nagai et al. 2011a). During the journey of asbestos fiber in the lung to the pleural cavity, they collect iron from hemoglobin and other proteins on the surface. Those are famous hallmark of asbestos exposure called asbestos body, which is asbestos fiber coated with massive iron (Fig. 19.5). Mesothelial cells have phagocytic activity in continuation with lymphatic vessels. Finally, those iron-coated asbestos fibers are taken up by the parietal mesothelial cells, and fibers tangle with chromosomal structure of mesothelial cells, which are thought to induce mutations in the mesothelial cells (Toyokuni 2009a).

There are characteristic mutational patterns in malignant mesothelioma: homozygous deletion of $p16^{INK4A}$ tumor suppressor gene, inactivation of Hippo pathway including *NF2* tumor suppressor gene, and inactivation of *BAP1* tumor suppressor gene (Chew and Toyokuni 2015). Malignant mesothelioma is divided into epithelioid, sarcomatoid, and biphasic (both epithelioid and sarcomatoid) subtypes, where sarcomatoid subtype reveals the worst prognosis and ~100% incidence of homozygous deletion of $p16^{INK4A}$ tumor suppressor gene (Oury et al. 2014). This fact is used

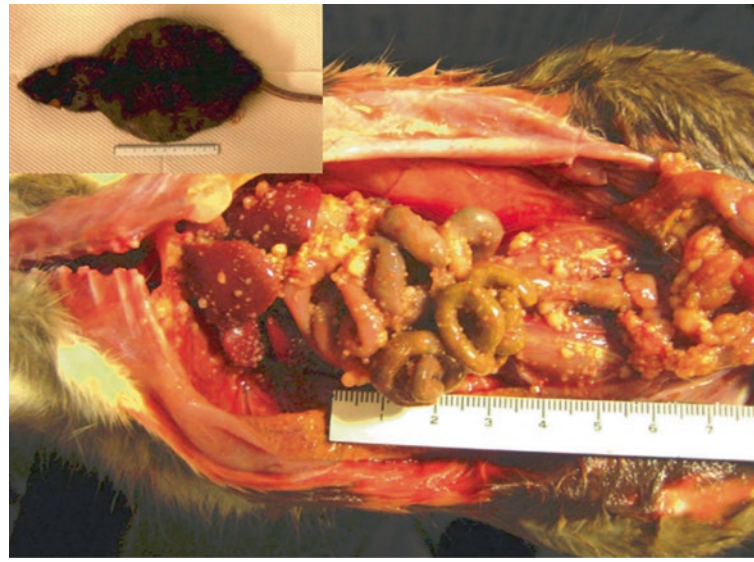

Fig. 19.6 Peritoneal mesothelioma induced by intraperitoneal administration of chrysotile (white asbestos; Jiang et al. 2012)

for the diagnosis of malignant mesothelioma with fluorescent in situ hybridization (FISH) analysis (Hwang et al. 2016), and the same mutation occurs in a rat model by intraperitoneal administration of asbestos fibers (Fig. 19.6) (Jiang et al. 2012). Of note, if mesothelial cells are directly exposed to asbestos fibers, only 1.5 years is necessary to induce malignant mesothelioma in virtually all the rats. There was some warning from the scientists that some carbon nanotubes (synthetic nanomaterial) but not all of them have the same capacity for mesothelial carcinogenesis as asbestos (Nagai et al. 2011b). Therefore, ample care has to be taken for the workers dealing with those nanotubes.

19.4 Prevention

What happens if Fenton reaction is repeated many times in an organ *in vivo*? Dr. Shigeru Okada and Prof. Osamu Midorikawa found the answer in 1982 with serendipity (Ebina et al. 1986; Toyokuni 2016). With repeated intraperitoneal injections of an iron chelate, ferric nitrilotriacetate, Fenton reaction was unexpectedly induced in the renal proximal tubules, which eventually led to a high incidence of adenocarcinoma (Fig. 19.7), originating from renal proximal tubules (Toyokuni 2016). This is distinct in that (1) most of the tumors induced by this way were highly aggressive despite the fact that only wild-type animals were used and (2) genomic alterations induced here were very similar to those in human cancers and showed a high incidence of homozygous deletion of $p16^{INK4A}$ tumor suppressor gene, suggesting that this is a footmark of excess iron-induced carcinogenesis (Akatsuka et al. 2012).

Iron has been a very precious metal for all the lives on earth. Independent life on earth should have been born with the reaction with iron. Therefore, we do not have any active pathway to excrete iron once it is absorbed through duodenal villous glandular cells into the blood (Toyokuni et al. 2017). Therefore, the only way to remove iron from our body would be phlebotomy or blood donation. Phlebotomy has been used as an official therapy in Japan for chronic active hepatitis by hepatitis C virus when other therapies did not work and showed effects to decrease activity of hepatitis and an incidence of hepatocellular carcinoma (Kato et al. 2007).

Because major infectious diseases, such as tuberculosis, have been conquered during the latter half of the twentieth century, now the tear and wear by the use of iron and oxygen is an unavoidable process in our body, which eventually causes human mortality. Most probably, the major factor to cause mutations in our genome is iron and

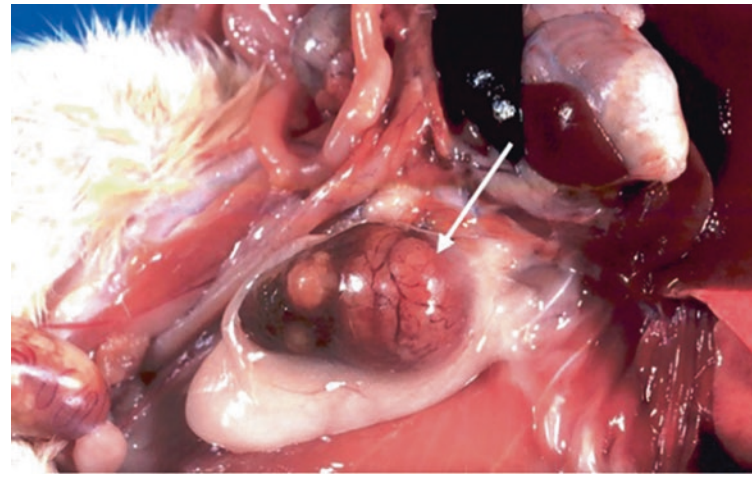

Fig. 19.7 Ferric nitrilotriacetate-induced renal cell carcinoma in a rat. Whitish mass (arrow) is the renal cell carcinoma in a rat kidney

oxygen, which is the major pathology of carcinogenesis with aging (Toyokuni 2011; Toyokuni et al. 2017). Recently, we successfully prolonged the survival and reduced the tumor mass of animals with phlebotomy in crocidolite-induced mesothelial carcinogenesis as a preclinical experiment (Ohara 2018; Toyokuni 2018).

Questions

1. Why is iron excess associated with carcinogenesis?
2. Why is asbestos fiber carcinogenic to humans?
3. Give another example of iron-induced carcinogenesis in humans, and explain the mechanism.

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