



Cancer wars: significance of protein unfolding in cancer and its inhibition with natural amphiphilic substances

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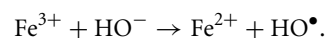
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It is essential to bear in mind that the native conformation of human proteins is stabilized by *intra*-molecular disulfide (S–S) bonds between a single or multiple polypeptide chains. The formation of S–S bonds is catalyzed by protein disulfide isomerase (PDI) (1), the activation of which is associated with a number of human diseases, such as myocardial infarction, stroke, and cancer. Unless proper chaperone proteins are available the accidental cleavage of S–S linkages will result in the unfolding and scrambled refolding of the polypeptide chains thus producing non-native species present in many degenerative diseases (2–5). This process is the most common protein post-translational modification that, for example, in a protein with 9 disulfide bridges can theoretically form 34,459,425 different disulfide-bonded isomers, only one with a correct protein function. However, the most damaging consequence of such a modification is the formation of protein aggregates known as “inclusion bodies” that are resistant to the enzymatic degradation (6). This unusual phenomenon is the result of the formation of *inter*-molecular hydrophobic bonds, which in contrast to peptide links are not susceptible to the catalytic hydrolysis. It is known that the strongest and practically irreversible protein interactions involve hydrophobic bonds, which in native proteins are buried inside their tertiary structure (7).

One of the blood proteins rich in disulfides is fibrinogen (Fbg), the physiologic function of which is to provide hemostatic fibrin plug formed by the action of enzyme thrombin. This insoluble polymer, when formed at the site of vessel wall injury, is eventually removed by the

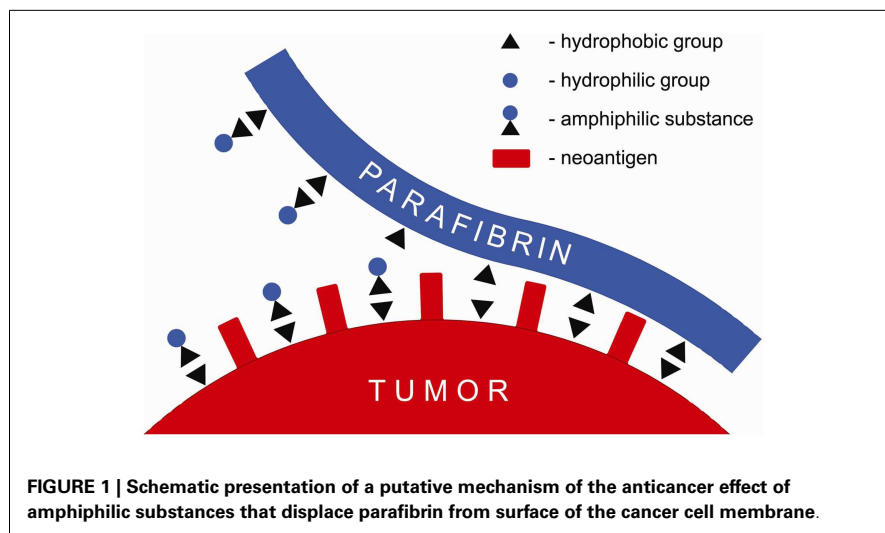
action of fibrinolytic enzyme system, to give space for the growth of a connective tissue and to ensure proper wound healing. To speed up the process of fibrin elimination from the coronary or cerebral circulations, several thrombolytic therapies have been devised with the use of a variety of fibrinolytic agents. It is, however, well recognized in clinical practice that such therapies are effective only when installed 3–5 h after the onset of thrombosis (8). This enigma is now resolved by the discovery of the alternative pathway of blood coagulation induced with iron (9). Thus, in contrast to thrombin-generated fibrin the iron-induced *parafibrin* is totally resistant to the fibrinolytic degradation. This is due to the fact that parafibrin has different a tertiary structure than fibrin formed with thrombin. We have showed that such a dramatic modification of fibrin structure is due to the unfolding and scrambled refolding of Fbg disulfide-linked subunits leading to the exposure of hydrophobic epitopes in their polypeptide chains (9). The cleavage of disulfide bonds is induced by biologically highly reactive hydroxyl radicals (HO[•]) formed in the reaction between trivalent iron with hydroxyl groups of water according to the following formula:



As the consequence of the hydroxyl radical interaction with Fbg a huge protease resistant polymer is formed that remains in the circulation for a long time, resulting in a state of chronic inflammation due to the attraction of cytotoxic albeit ineffective T cells. The accumulating evidence

indicates that there is a correlation between increased blood concentration of unbound iron and the incidence of cancer in humans (10–13), and that its reduction may prevent cancer morbidity and mortality (14). It should be noted that it is only the trivalent iron (Fe³⁺), and not divalent (Fe²⁺), which participate in the generation of hydroxyl radicals and subsequent formation of insoluble parafibrin from soluble plasma Fbg. However, when hemoglobin is released from the hemolyzed erythrocytes, the divalent ferrous ions are enzymatically converted to ferric ions. Thus, any pathologic condition in which erythrocyte membranes are damaged, e.g., in infections and/or after exposure to environmental toxins, may contribute to the excessive body storage of trivalent iron. It should be borne in mind that this form of iron accumulates with age due to the fact that there is no mechanism for its physiologic elimination, and may therefore, explain association of cancer with aging.

The unsuccessful attempts at removing parafibrin by the human body defense systems were recently suggested to contribute to Alzheimer's disease (15) as well as to the cardiovascular disease (16). These diseases and other degenerative disorders have been known to respond well to dietary modifications, particularly to those associated with the so-called Mediterranean diet (17), which is rich in natural amphiphilic substances such as polyphenols and flavones. Relevant to the concept presented in this article is the fact that protein unfolding can be inhibited by natural products present in tea, fruits, berries, and certain grains, the consumption of which is known to lower the incidence of degenerative



diseases (18). In addition, there is another important component of human diet, selenium, which is known to prevent various forms of cancer (19–23). Hence, sodium selenite, but not selenate, reacts with free sulfhydryl groups of proteins, thus preventing reductive cleavage of disulfide bonds followed by protein unfolding and abnormal refolding (24, 25).

It is proposed in this paper that the barrier formed around tumor cells composed of proteolytically resistant parafibrin can be removed by a non-enzymatic mechanism based on the interaction of hydrophobic and hydrophilic groups (Figure 1). Numerous natural substances, particularly those of amphiphilic nature such as polyphenols, when ingested with diet in sufficient quantities can prevent and/or reverse cancer formation and metastases (26–33). These findings may explain beneficial effects of the Mediterranean diet known to be associated with lower incidence of cancer and other degenerative diseases (34).

According to the mechanism shown in Figure 1 amphiphilic substances taken up with food interfere with and/or displace the hydrophobic coat on cancer cells membranes thus exposing neoantigens and making tumors vulnerable to the natural killer cells attack and lysis.

In conclusion, the concept delineated in this article supports the original notion expressed by Basmadjian et al. (35) according to which natural products have a potential to be developed into novel anticancer medicines.

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