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Changes in Glutathione Activity in Pathophysiology of Reproductive Organs Cancers

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It is an established fact that oxidative stress, caused by an imbalance between prooxidants (such as reactive oxygen species (ROS)) and antioxidants, plays an important role in carcinogenesis of reproductive organs' cancer. Many studies have investigated the presence of antioxidants and their transcripts in the female reproductive tract indicating that the balance between ROS and antioxidants greatly influences the reproductive activities in females, such as endometrial changes in different luteal phases, folliculogenesis, ovulation, fertilization, placental growth, embryogenesis, and implantation [20]. Thus, antioxidants are critical for maintaining the redox balance in the ovaries and uterus to support normal reproductive function. However, their exact molecular mechanisms and roles during carcinogenesis have not been fully elucidated yet.

GSH is considered the major representative of nonenzymatic antioxidants present in oocytes, embryos [20] and uterus as well. GSH is involved in many cellular functions, including cell proliferation, differentiation, and apoptosis [20]. The studies on ovarian physiology have shown that the increase in steroid production in the growing follicle causes an increase in P450, resulting in ROS formation. Follicular ROS promotes apoptosis, whereas GSH and follicular stimulating hormone (FSH) counter balance this action in the growing follicle [1]. However, high intracellular GSH levels are important contributors to pathologies such as, cellular transformation and resistance to radiation and antineoplastic treatments in cancer cells [15, 18].

Many antineoplastic agents, in particular the alkylating agents, are designed to modify DNA and react with electrophilic sites. Glutathione therefore is thought to intercept and inactivate these agents before they can act to kill the cancer cell. Furthermore, GSH is known to mediate resistance to both cisplatin and carboplatin through several mechanisms such as drug uptake reduction and increased intracellular drug detoxification/ inactivation increased DNA repair and inhibition of apoptosis drug-induced oxidative stress [5]. Specifically in ovarian cancer, although with some controversy, several reports

already associated high GSH levels or glutathione S-transferase P1 (GSTP1) activity with cisplatin or carboplatin resistance. High levels of GSH are required in well-oxygenated normal tissue to protect the cells against oxidative stress, since competing reactions of GSH are very rapid. This suggests that low to moderate decrease in GSH contents will have little effect on the radiosensitivity and chemosensitivity of normal cells. In contrast, under low oxygen tensions (hypoxia), such as those that are frequently seen in tumor tissues, GSH may play a dominant protective role. Therefore, depletion of GSH in the hypoxic tumor tissues will make them more susceptible to radiotherapy and chemotherapy [5].Thus, in the context of altering the tumor microenvironment a systematic understanding of GSH fluctuations pattern, as a major non enzymatic antioxidant in reproductive organs, is required.

Recently, we have shown that changes in Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin (CN) activity in the pathophysiology of ovarian and uterine cancer depends on the stage of the disease [8, 12]. Expression and activity of CN are modulated by intracellular redox status. GSH has been shown to promote CN phosphatase activity [2]. Therefore, taking into account all of above mentioned, in this study, we have considered advisable to investigate also the changes in GSH activity in plasma and tumor tissue samples of previously untreated patients with primary ovarian and uterine cancer depending on the stage of disease.

Material and Methods

Sample collection and preparation. The blood and tissue samples from postoperative material of untreated patients with the I (n=6), II (n=7) and III (n=5) stages of primary ovarian cancer and I (n=17), II (n=8) and III (n=8) stages of primary uterine adenocarcinoma were provided by the National Centre of Oncology after V.A. Fanarjyan (NCO MH RA). The plasma of healthy donors (n=6) and histologically checked healthy parts of remote tissue (n=8 for uterine and n=4 for ovaries) were used as a control. The protocol was approved by the Research-related Ethics Committee of the H. Buniatian Institute of Biochemistry NAS RA (IRB0001621, IORG 0009782, Ref. let. #5). Histological study of the postoperative material was conducted by the Laboratory of Clinical Pathomorphology at the NCO MH RA. The most cases of ovarian cancer were diagnosed as a moderately and poorly differentiated adenocarcinoma. Age of patients ranged from 45-80, and the average age was 61 years. In case of uterine carcinoma, moderately differentiated endometrioid adenocarcinoma was diagnosed mostly. Age of patients ranged from 35-76, and the average age was 61 years as well.

Blood (1.5-2 ml) was collected into sodium citrate (3.2%)-coated vacutainer tubes and centrifugated at 1500 rpm for 10 min. Plasma was separated and stored at -32°C. Tissue samples were homogenized with 5

volumes of 50 mM Tris-HCI, pH 7.5 buffer, containing 0.05% Triton-X-100, 0.1 mM EDTA, 1 mM ditiothreitol (DTT), protease inhibitors, and centrifugated at 20000×g for 60 min at 4ºC. The supernatant was separated and stored at -32°C as well.

The protein content in samples was determined by Bradford assay [4].

Glutathione activity. The activity of GSH was studied using DTNB/GR enzyme recycling method [16]. GSH is oxidized by 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) resulting in the formation of GSSG and 5-thio-2-nitrobenzoic acid (TNB). GSSG is then reduced to GSH by glutathione reductase using reducing equivalent provided by NADPH. The rate of TNB formation is proportional to the sum of GSH and GSSG present in the sample and is determined by measuring the formation of TNB at 412 nm using LABLine-022 microplate reader (LABLINE Diagnostics, Austria).

Statistical analysis. Experiments were performed at least three times. The results were expressed as the means ± SEM. Statistical analyses were performed using Origin 6.1 software. Statistical significance was defined at $p<0.05$ and was determined with one-way ANOVA.

Results and Discussion

Results obtained revealed that plasma GSH activity of the both ovarian and endometrial cancer patients changed in a parallel manner (Fig. a, b). It was demonstrated dramatic 2.9 fold and 2.8 fold decreases in ovarian and uterine cancer patients respectively, for the I stage of disease compared with the control groups. The significant increase was found in the II (2.4 fold and 4.7 fold, respectively) and III stages of disease (5.6 fold and 5.2 fold, respectively) compared with the I stage. In uterus tumor tissue GSH activity demonstrated the same picture as in plasma. We have found statistically significant 2.3 fold decrease in the I stage of disease compared with the control group (Fig. a). In

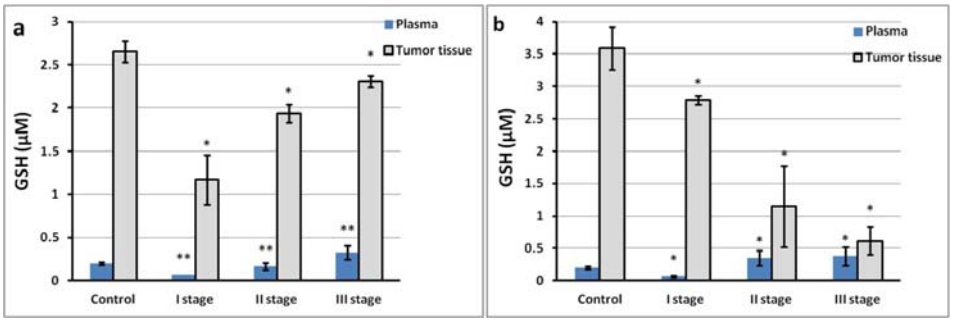

Fig. GSH activity in plasma and tumor tissue of the primary uterine (a) and ovarian (b) cancer patients with the I, II and III stages of disease. * p<0,05; **p<0,01 for the I stage compared with control, and for the II and III stages compared with the I stage (one-way ANOVA)

the II and III stages of disease it demonstrated 1.7 fold and 2 fold increases respectively, compared with the I stage. As one can see from Fig.1b, in ovarian tumor tissue GSH activity was shown to be decreased in parallel with the stages of disease: 1.3 fold in the I stage of disease compared with the control and 2.4 fold and 4.6 fold in the II and III stages of disease, respectively, compared with the I stage.

Glutathione is a ubiquitous tripeptide that functions as an important intracellular radical scavenger. It protects cells against ROS, as well as against many toxins, mutagens, and drugs [11]. Recent studies have demonstrated that GSH levels are elevated in various human cancer tissues including breast and ovary, as compared with normal tissue in these regions. It was reported that elevated GSH levels in tumor tissue are associated with resistance to chemotherapy [13].

It is well known that tumor milieu is enriched with ROS with their huge impact on cancer initiation and progression [8]. However, in cancer cells, ROS is hold at low to moderate levels to induce cell proliferation and cell survival, and to avoid high level induced cell damage and at an excessive level induced cell death [9]. Being the major intracellular antioxidant, GSH could undergo a sharp depletion for the initial stages of cancer progression as a major adapting factor for cancer cells to regulate and limit oxidative stress. Furthermore, the gradual increase of the GSH activity in the II and III stages of disease could be explained by the recovery of GSH recycling and recovering systems, mainly by glutathione reductase (GR). GR is considered as a central enzyme of cellular antioxidant defense, as it catalyzes the reduction of oxidized glutathione disulfide (GSSG) to the sulfhydryl form, glutathione. Recently Ana Todorovi´c et al. have shown that compared to patients with polyps and myoma, levels of GR protein and GR mRNA were elevated in women with both hyperplasia and adenocarcinoma [17]. It is known that many types of treatment resistant malignant tumors are characterized by the increased expression of enzymes involved in the GSH metabolism [3]. Thus, indirectly, our results also pointed out the possible therapy resistance of uterine adenocarcinoma, as the high level of GSH was previously recorded in some radio- or chemoresistant cancers, such as breast tumor, melanoma, and lung cancer [17].

The finding that stage dependent changes of GSH activity in plasma and tumor tissue of the ovarian cancer patients was not parallel, probably, indicates existence of difference in schemes of regulation. Fluctuations of FSH expression and activity along with the differences in stage dependent changes between levels of systematic circulating estrogen and its local counterparts is among factors that most probably could explain obtained data. It is well documented that in ovaries FSH interacts with estrogen to regulate the synthesis of GSH [6]. Moreover, level of circulating FSH in women of postmenopausal age is quite elevated [19], which is in consistent with our data regarding GSH plasma levels in ovarian cancer.

More recently, we have shown that CN activity in plasma and tumor tissue of patients with primary ovarian and uterine cancer changes depending on the tumor stage of the disease [8, 12]. CN widely involved in signaling pathways of the immune response and cancer [7, 14]. GSH is known to protect disulfide bonds of CN from ROS attacks via reduction of oxidized ones and, thus, protect CN from inactivation [10]. There is need for additional studies to explain the finding that changes in CN and GSH activities in reproductive organs cancer were not parallel.

In conclusion, in this study we have shown that GSH activity in gynecological cancers changes depending on the stage of disease.

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Գլուտաթիոնի ակտիվության փոփոխությունը վերարտադրողական օրգանների քաղցկեղի պաթոֆիզիոլոգիայում

Գ. Ա. Հովհաննիսյան

Վերջերս ցույց ենք տվել Ca^{2+}/CaM-կախյալ պրոտեին ֆոսֆատազ կալցինեյրինի (CN) ներգրավվածությունը ձվարանների և արգանդի քաղցկեղի պաթոֆիզիոլոգիայում և պարզել ենք, որ քաղցկեղով հիվանդների պլազմայի և ուռուցքային հյուսվածքներում CN-ի ակտիվության փոփոխությունները կախված են հիվանդության փուլից: CN-ի էքսպրեսիան և ակտիվությունը պայմանավորված են ներբջջային օքսիդավերականգնողական կարգավիճակով: Ցույց է տրվել, որ գլուտաթիոնը (GSH), որը բջջի հիմնական հակաօքսիդանտներից է, նպաստում է CN-ի ֆոսֆատազային ակտիվությանը: Բացի այդ, GSH-ը սերտորեն կապված է գինեկոլոգիական քաղցկեղի բուժման հիմնական մեթոդների հանդեպ քիմիո-և ռադիոկայունության դրսևորման հետ: Այս աշխատանքում ուսումնասիրել ենք GSH-ի ակտիվության փոփոխությունը ձվարանների և արգանդի առաջնային քաղցկեղով չբուժված հիվանդների պլազմայում և ուռուցքային հյուսվածքում: Արդյունքները

ցույց են տվել, որ GSH-ի ակտիվության փոփոխությունները նույնպես կախված են հիվանդության փուլից: Ստացված տվյալներն ընդլայնում են գիտելիքները գինեկոլոգիական քաղցկեղի զարգացման ընթացքում օրգանիզմի հակաօքսիդանտային ակտիվության փուլից կախված փոփոխությունների ներգրավման մասին:

Изменения активности глутатиона в патофизиологии рака репродуктивных органов

Г.А. Оганисян

Недавно нами получены данные об участии Ca^{2+}/CaM-зависимой протеинфосфатазы кальцинейрина (CN) в патофизиологии рака яичников и матки, а также показано, что изменения активности CN в плазме и опухолевой ткани онкологических больных зависят от стадии заболевания. Экспрессия и активность CN модулируется внутриклеточным окислительно-восстановительным статусом. Было показано, что глутатион (GSH), один из основных клеточных антиоксидантов, способствует фосфатазной активности CN. Кроме того, GSH тесно связан с химио- и радиорезистентностью к основным методам лечения гинекологического рака. В данной работе мы изучили изменения активности GSH в образцах плазмы и опухолевой ткани ранее нелеченых пациентов с первичным раком яичников и матки. Полученные результаты показали, что изменения в активности GSH также зависят от стадии заболевания. Полученные данные расширяют знания о причастности стадийно-зависимых изменений антиоксидантной активности организма при прогрессировании гинекологического рака.

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