Chapter 3

Role of Endocrine-Genotoxic Switchings in Cancer and Other Human Diseases:
Basic Triad

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Abstract

Cancer is one of the leading causes of human death and belongs to the group of main chronic noncommunicable diseases (NCD). Certain specific features of NCD have raised the concept of 'normal' and 'successful' aging. The apparent paradox of simultaneous increase with aging of the diseases connected with estrogen deficiency as well as with estrogenic excess can be explained by the existence of the phenomenon of the switching of estrogen effects. An isolated or combined with the weakening of hormonal effect increase in genotoxic action of estrogens can modify the course of age-associated pathology. In particular, such changes in estrogen effect may alter the biology of tumors to make them less favorable/more aggressive. Two other endocrine-genotoxic switchings (EGS) involving phenomena of Janus (dual) function of glucose and adipogenotoxicosis may produce similar influences on tumor and other NCD biology. These three phenomena form a 'basic triad' and can act independently of each other or in concert. EGS and their inductors may serve as targets for prevention and, probably, treatment of main noncommunicable diseases. The measures to correct components of the 'triad' can be divided into several groups aimed to optimally orchestrate the balance between endocrine and DNA-damaging effects of estrogens, glucose and adipose tissue-related factors.

Introduction

Cancer (including tumors of hormone dependent tissues) is one of the leading causes of human death and belongs to the group of main chronic noncommunicable diseases (NCD).[1,2] In addition to cancer, several NCD such as atherosclerosis, arterial hypertension, diabetes, neurodegenerative pathology, chronic pulmonary disease and osteoporosis increase with advancing age.[2] The burden of these diseases, as is well known, becomes particularly prevalent in the second half of life, after age 50-60. Notwithstanding the quite demonstrative increase in the average age when these diseases are diagnosed, the characteristics of them including clinical course and individual time of onset are highly variable. Under the surface of the same nosological form can be hidden distinctive pathological processes, which result in differences in aggressiveness, alterations in the frequency of their appearance in the population, changes in the rate/velocity of progression and reaction to treatment. In aggregate, these distinctions may lead to different levels of mortality and—as reflection of that—to individually different life span.

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In the attempt to explain such widespread and not rarely discussed differences in morbidity and mortality at the later stages of ontogenesis, the concept was proposed that aging could either be normal or successful.[3,4] According to this concept[4] successful aging was characterized by three components: (1) (relatively) high mental and physical function, (2) active engagement with life, including close relationships with others and most importantly (3) (relatively) low risk of disease and disease-related disability. With complete understanding that the term successful aging may itself have the unintended effect of defining a significant part of population as unsuccessful and therefore as failing, the authors hoped that their classification will invite researchers to investigate the heterogeneity among middle-age and older people and to discover its causes—genetic, psychosocial and environmental.[5]

The possible causes of these distinctions are not, of course, limited to those presented above. The key applicable word is "heterogeneity" and this applies to the mechanisms of hormonal carcinogenesis (see in detail the chapters by Chen and Yager and Santen et al in this volume) as well as to the factors predisposing to the two principal types of the latter—promotional or stochastic/mitogenic and genotoxic.[6-9]

During several recent years we have attempted to understand what conditions may advance or be associated with a shift from promotional to less favorable genotoxic type of hormone-induced carcinogenesis and from less to more aggressive variants of several other noncommunicable diseases. Subsequently, we have focused upon three events: phenomenon of switching of estrogen effects (PSEE), Janus, or dual, function of glucose (JFG) and adipogenotoxicosis (AdG).[10-12] This treatise will first provide an introductory background and then, present an analysis of these phenomena (forming so called 'basic triad') as well as the practical implications following from them.

General Principle: Types of Effects

The world rather often is binary. Although transitions from the one state to another sometimes are not possible, in fact, in biology and medicine almost nothing goes according to one scenario. Even in seemingly very strict situations, like cell fate determination involving Notch signaling, all cells in a given population can adopt an alternative fate, but some maintain this new destiny stably, whereas others revert to the default state.[13] In case of the whole organism choices are understandably manifold and variable. Nevertheless, with reference to hormones and some hormone-associated substances their different activities (taking into account possible associations with cancer and other NCD) may be reduced to the two primary: hormonal or endocrine and DNA-damaging or genotoxic.[9,10] Factors inducing or supporting an increase in the ratio of genotoxic/hormonal effects can be considered correspondingly as direct or indirect genotoxicants. Endocrine-genotoxic switchings (transitions which alter function in the direction of DNA-damaging effects) may be not only induced but also spontaneous/constitutive, e.g., in genetically or otherwise predisposed persons. These switchings can be manifested by a) an isolated increase of genotoxic effects without a decrease in hormonal effect (relative predominance) as well as with b) combined trend toward an increase in genotoxic effects and decrease in hormonal effects (absolute predominance), Figure 1. Understandably, it is not enough to admit that the coin has two sides; depending of situation it is essential also to clarify which side and when is more important.[14]

Phenomenon of Switching of Estrogen Effects (PSEE)

Although an idea that estrogen-induced carcinogenesis per se and the modulating action of estrogens on carcinogenesis are different notions was emphasized a rather long time ago,[15] drawing a line between these two events is not very easy. One explanation for such difficulty lies in the absence of complete understanding of whether the modifying effect of hormones involves only epigenetic pathways.[16]

Those who believe in the exclusive role of estrogens as mitogenic and promoting factors proposed that increased hormonal stimulation is an important link in the process of hormone dependent tumor development. The attention of these investigators was attracted first by the observation of enhanced proliferation in target tissues under conditions of excessive estrogenic influence.

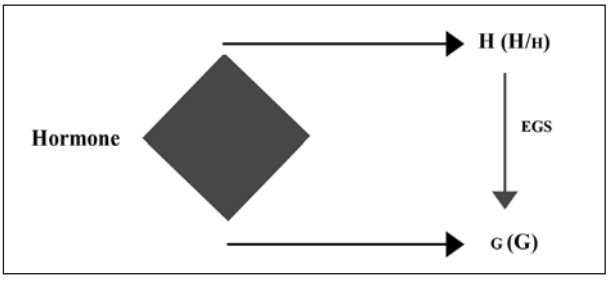

Figure 1. The principle of endocrine-genotoxic switchings applied to hormones, hormone-related substances and changes in target tissues. H—hormonal effect (not changed or decreased); G—genotoxic effect (increased); EGS—spontaneous or induced endocrine-genotoxic switchings.

Thus, in other words, it was concluded that "no increased proliferation—no hormone-induced carcinogenesis".

Later studies stated that though "... proliferation is necessary, it may not be sufficient for neoplastic transformation"[6,17] and suggested several additions to the proffered scheme. This, after passing through critique, discussions and several intermediate concepts,[16] led finally to contemporary point of view which indicates that mitogenic as well as mutagenic effects of estrogens act in concert to initiate and promote the development of cancer.[6,8,18] Initially, rather extensive research has concentrated upon possible procarcinogenic properties of 16α-hydroxylated estrogens, including their genotoxic effects.[19] More recently, 4-hydroxylated metabolites of estradiol and estrone and their further metabolic conversions, in preference to the 2-hydrohylated derivatives, have been the focus of studies in the context of estrogen-induced cancer.[7,20,21] Additionally, recent observations demonstrated the ability of estradiol to activate human CYP1B1 (estrogen-4-hydroxylase) gene via ER-alpha, thereby providing insights into the homeostasis of estrogen metabolism as well the interaction of potential pathways of estrogen-induced carcinogenesis.[22]

The dual role of estrogen in tumor development as both a hormone stimulating cell proliferation and as a procarcinogen that induces genetic damage, indicates that the search of conditions or factors modulating the genotoxic component in total estrogenic (genomic and nongenomic) effect can be essential. Such factors may influence both the hormonal carcinogenesis process and biological properties of the developing hormone-dependent tumors.[9,23]

As we demonstrated previously, in oophorectomized rats, tobacco smoke induces phased changes in uterotrophic action of estrogens, which finally result in attenuation of the hormonal (H) component of their effect (dynamics of uterine weight and proliferation index, etc) and in increase of the rate of genotoxic (G) damage (COMET assay). The phenomenon was referred as switching of estrogen effects (PSEE).[10,24] Interestingly, although it is known that smoking increases 2-hydroxylation of estrogens[25] the same factor may stimulate CYP1B1 in the aerodigestive tract,[26] thus denoting one of the pathways through which estrogenic DNA-damaging activity can be increased.

In a recently performed investigation, the hormonal and genotoxic effects of estradiol (E2) and their possible modification by diluted tobacco smoke condensate (TSC) were studied in breast cancer MCF-7 cell line.[27] TSC decreased effect of E2 on the cell counts and opposed the anti-apoptotic influence of this hormone (Fig. 2A,B). The combination of TSC with E2 promoted progesterone receptor B induction after 5 days of cocultivation (Fig. 2C). However, in long-term (3 mo) studies in vivo the same combination of agents led to a diminution of this hormonal estrogenic effect.[24] In addition, in MCF-7 cells treated with TSC and E2 (in the lesser of two studied concentrations, 10^{-11} M) the immunocytochemical staining of oxidative DNA damage marker, 8-OHdG, revealed higher values than in cells processed with these agents separately (Fig. 2D).[27]

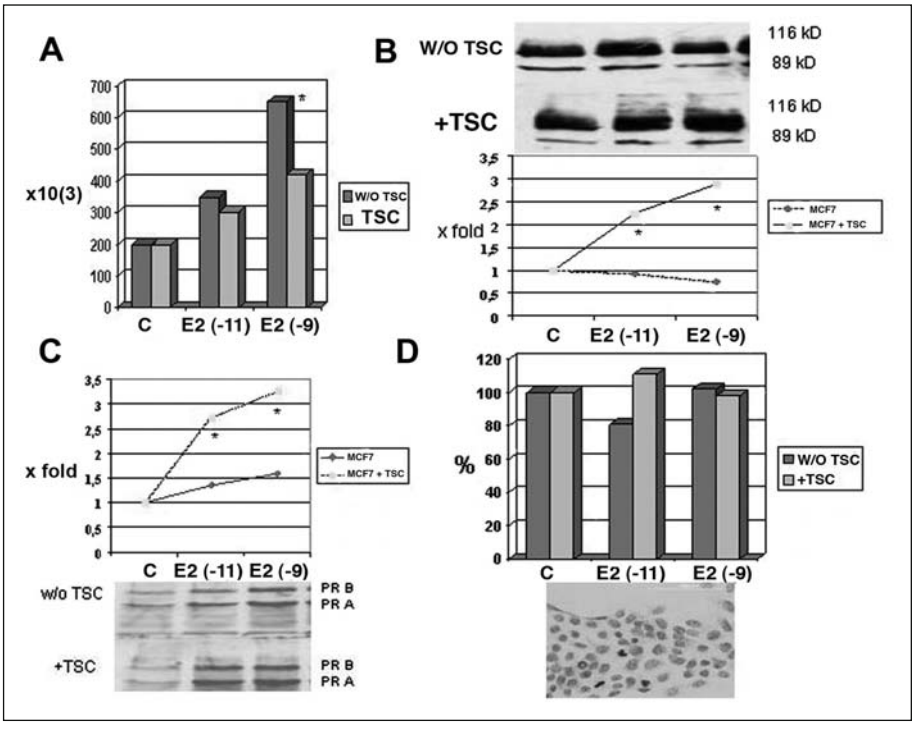

Figure 2. Modification of estradiol (E2) effects by tobacco smoke condensate (TSC) in MCF-7 cells. Diluted TSC was used in final concentration equivalent to 2.5 mcg of cigarette tar/ml medium and E2 as 10^{-11} M and 10^{-9} M. The duration of experiments varied between 2 and 5 days. A) Cell counts. Cell growth was monitored by cell number counting. Briefly, cells growing in 6-well plates were treated with E2 and/or TSC as indicated in figure legend. Cells were rinsed with 0.9% saline, lysed in ZAP buffer and counted with a model Z1 Coulter counter. B) Apoptosis. This parameter was evaluated by the cleavage of poly-ADP ribose polymerase (PARP) and immunoblotting. Cells were washed with ice phosphate-buffered saline and extracted with binding buffer. Equal amounts of protein from cell extracts were analyzed on SDS-polyacrilamide gels and transferred to PVDF membranes. The membranes were probed with rabbit polyclonal antiPARP antibody, 1:1000 (Cell Signaling). The immunoblots were incubated with antirabbit secondary antibodies and further developed using the chemiluminescence detection system (Pierce). Data are presented as 116 kD/89 kD ratios. C) Progesterone receptor (B isoform). In this case PVDF membranes were probed with mouse monoclonal antiprogesterone receptor antibodies, 1:1000 (Cell Signaling). The immunoblots were incubated with antimouse secondary antibodies and developed using the chemiluminescence detection system. Data are presented as PR B ratio in relation to control values. D) 8-hydroxy-2′-deoxyguanosine (8-OH-dG). Cells were fixed with ethanol and acetone and evaluated by immunocytochemical method with mouse monoclonal anti-8-OH-dG antibody 4E9, 1:200 (Trevigen). Data are presented as relative scores.

The latter data are in line with observations demonstrating higher levels of DNA adducts in cervical tissue of young smoking women taking oral estrogen-containing contraceptives in comparison with nonsmokers or contraceptive nonusers.[28] They also correspond with data reporting higher excretion of carcinogenic and genotoxic cathecholestrogens (in particular, 4-hydroxyestrogens) in smoking postmenopausal women treated with estradiol valerate, Proginova (Table 1).[29] It is noteworthy that before the start of estrogen replacement therapy, the excretion of 4-hydroxyestrone and 4-hydroxyestradiol in smokers was not higher than in nonsmokers. This observation suggests that

Table 1. Urinary excretion of estrogen metabolites as means and standard deviations, nmol/24 h in nonsmoking and smoking postmenopausal women receiving estrogen replacement therapy, ERT

	Nonsmokers		Smokers	
Urinary Estrogen Fraction	**Before ERT**	**After ERT**	**Before ERT**	**After ERT**
E1	9.70 ± 8.60	1232.6 ± 295.2	14.46 ± 13.24	951.5 ± 181.2
E2	2.87 ± 2.14	212.6 ± 42.2	3.65 ± 2.60	235.7 ± 40.5
E3	4.38 ± 2.37	178.3 ± 75.2	3.31 ± 2.49	131.2 ± 83.2
4-OHE1	2.29 ± 1.28[a]	28.8 ± 10.6	1.09 ± 0.51[a]	43.7 ± 18.2
4-OHE2	0.07 ± 0.08	23.5 ± 15.6[a]	0.10 ± 0.13	60.8 ± 25.3[a]
2-OHE1	8.88 ± 8.42	226.9 ± 68.3[a]	6.52 ± 3.78	330.9 ± 80.0[a]
2-OHE2	2.19 ± 2.09	36.4 ± 11.7	2.05 ± 0.56	45.8 ± 9.2
2-MOE1	2.49 ± 1.57	53.4 ± 15.1	2.36 ± 0.79	51.3 ± 21.9
2-MOE2	0.37 ± 0.26	3.10 ± 0.80	0.41 ± 0.25	3.20 ± 1.10
16α-OHE1	3.79 ± 3.40	104.8 ± 78.1	2.46 ± 1.40	105.8 ± 66.8

[a]Difference between smokers and nonsmokers is significant (p at least <0.05). E1, estrone; E2, estradiol; E3, estriol; 4-OHE1, 4-hydroxyestrone; 4-OHE2, 4-hydroxyestradiol; 2-OHE1, 2-hydroxyestrone; 2-OHE2, 2-hydroxyestradiol; 2-MOE1, 2-methoxyestrone; 2-MOE2, 2-methoxyestradiol; 16α-OHE1, 16α-hydroxyestrone. Reprinted from Berstein LM, Tsyrlina EV, Kolesnik OS et al. J Steroid Biochem Mol Biol 2000; 72:143-7. With permission from Elsevier.

only the combination of external estrogen and smoking promotes the switch into the direction of increased formation of genotoxic estrogen metabolites.[10,29]

For the subject under consideration and in addition to the named estrogen metabolism features, the sensitivity of target tissues to estrogens is without doubt of importance too. About 30-40% of breast cancers lack steroid receptors (ER and/or PR) at diagnosis, a finding which predicts an unfavorable prognosis and a limited response to usage of hormone therapy.

Opinions differ as to whether receptor negative cancers arise from R(–) compartment within the mammary epithelium or represent evolution from R(+) to R(–) state. Evidence in support of the idea on distinct etiologic pathways rather than different stages in the natural history of breast cancer has been recently growing.[30,31] Receptor-positive and receptor-negative breast cancer subtypes may have associations with distinctive risk factors and heterogeneity by hormone-receptor status related to initial existence of the two separate types of cancer (R+ and R–) is rather possible.[30,32]

The mechanisms leading to the development of receptor-negative breast cancer (BC) warrant further studies. Existing interpretations are not definitive and can be reduced to the role of several genetic (including BRCA1 and BRCA2) and epigenetic factors, interrelations with the presence of EGF and erbB2/HER-2/neu receptors in tumor tissue and certain features of endocrine (reproductive) system which consider the level of estrogen in the blood and intratumoral aromatase/estrogen synthetase activity.[31,33-35]

Taking into account the principal characteristics of the phenomenon of switching of estrogen effects (PSEE) described above, the assumption has been made that weakening of hormonal and strengthening of genotoxic activity of estrogens may be of importance in predisposing to disturbances in estrogen signal transduction and preferential formation of receptor-negative BC.[24] In fact, statistically significant distinction between smoking and nonsmoking BC patients was revealed only in reproductive period and only in regard of ER+ PR– tumors, which were overrepresented in smokers ($t = 2.18$, $p < 0.05$; $\chi^2 = 5.01$, $p = 0.025$).[36] Predominant formation of the tumors with a phenotype presumably reflecting failure of estrogenic signal transduction and insufficient induction of estrogen-dependent proteins including PR[37,38] in smoking females with conserved

menstrual cycle, suggests that tobacco smoke promotes PSEE mainly in the case of excessive or at least nondeficient estrogenic stimulation.

A contemporary view links the origin of receptor-positive and receptor-negative breast tumors correspondingly with luminal and basal type of mammary epithelium.[39] Interestingly, the diminished survival observed in BC patients who smoke[40] can be associated with receptor-negative phenotype[36,41] and with switch to a basal/myoepithelial lineage. The same switch was discovered under influence of progestins which partly explains the higher BC risks and poorer prognosis on postmenopausal estrogen-progestin replacement therapy (HRT).[42] Effects of HRT differ depending on women age and are more favorable during a rather short (first 5-7 yrs after menopause) "critical window".[43] Thus it is possible that endocrine predominance in estrogen effect leads to less aggressive luminal subtype of BC, while genotoxic predominance in the action of these hormones, associated in particular with smoking, promotes more clinically tough and mostly receptor-negative mammary carcinoma type. Of note, in receptor-negative endometrial cancer type II, mutations of p53 are found[44] which arrest cellular check-points activation and promote proliferation of cells with signs of DNA damage.[45] Consequently, inefficient DNA repair may tentatively be included into the orbit of events directly or indirectly supporting a PSEE-associated genotoxic switch. Other genetic and epigenetic abnormalities related to DNA-damaging estrogen action may also be involved into this process.

We made an attempt to find additional factors inducing the phenomenon of switching of estrogen effects. The accumulated experimental data suggests that PSEE can be divided into complete switching with a simultaneous increase of genotoxic component and decrease of hormonal component in estrogenic effect and incomplete, with an isolated increase in DNA-damaging capacity only. The inductors of PSEE may be classified in a corresponding manner as complete and incomplete. Summing up results received in oophorectomized rats injected with estradiol, complete PSEE inductors include long-term treatment with tobacco smoke and drinking of 15% ethanol (i.e., in levels equal to chronic alcoholism). The group of incomplete inductors included consumption of alcohol in more moderate, 5%, concentration, single whole-body γ-irradiation in the lesser (0.2 Grays) of two investigated doses and aging.[10,24] Certain xenoestrogens may work in a similar fashion. Fortunately they are distributed in nature in low concentrations. Although their action may be mediated primarily through aryl hydrocarbonic receptors, or AhR,[46] this action may be estrogen-dependent as well. Indeed, dioxins induce DNA adducts formation in liver of intact but not oophorectomized rats[47] and in MCF-10A cells TCDD and estradiol do not provoke oxidative stress separately while induce it in combination.[48]

Thus, it is possible that low-concentrated but widespread progenotoxic "natural agents" combined with estrogenic stimulation might be a dangerous risk factor for cancer and some other chronic noncommunicable diseases. PSEE is manifested in such conditions as an increased genotoxic effect of estrogens, which may be or may be not coupled with retained hormonal, e.g., mitogenic, influence of these hormones.[9] The same rationale should be taken into consideration when analysis goes beyond estrogens.

Janus (Dual) Function of Glucose

Major investigative attention has focused during several recent decades upon the metabolic syndrome. The abnormalities associated with this syndrome, first of all hyperinsulinemia and insulin resistance, may increase the risk of hormone dependent cancer and predispose to simultaneous development of other frequent and, in the end, lethal chronic human illnesses (like cardiovascular disease, stroke, type 2 diabetes, etc.). Typical characteristics of the metabolic syndrome may include also visceral obesity, hypertension, chronic low-level inflammatory state, dyslipidemia and—rather often—impaired carbohydrate tolerance.[49-51]

Together with aging-related events, one of the greatest contribution to the current expansion of noncommunicable pathology is the combined influence of disordered nutrition and impaired physical activity.[1,2] In most parts of the world (understandably, with some exceptions), as food became more available, new insights into the relationship between carbohydrates and chronic diseases became apparent. A high glycemic load is associated with increased risk of these diseases

including cancer and a pathogenic role in the process can be played by postprandial hyperglycemia.[52,53] In concert with hyperinsulinemia and activation of IGF-I and mammalian target of rapamycin (mTOR) systems, glucose along with some other nutrients, creates a metabolic/mitogenic platform for the amplification of cellular proliferation.[54,55] Importantly, according to some observations, intrinsic or nutritionally induced glucose intolerance may increase risk of hormone dependent cancer to a higher degree than does overt diabetes.[56]

In mammalian cells the glucose fate begins with glucose transport and metabolism and ends, among innumerable other functions, with two actions, which can be considered as principal.[10,11,57] The first, which may be designated as an endocrine effect, is realized due to the ability of glucose to be a stimulus for hormonal secretion, particularly, for insulin production in pancreatic β-cells. The gradual β-cell failure, occurring as normal glucose tolerant individuals progress to type 2 diabetes, includes the whole group of different processes and reactions among which sensitivity of these cells to glucose, glucotoxicity and output of insulin are of primary importance.[58,59] The second principal function of glucose may be designated as a genotoxic, or progenotoxic. Oxidative damage to DNA is characteristic of overt diabetes mellitus[60] and hyperglycemia may contribute (under participation of mitochondrial electron transport chain) to the generation of oxidative stress resulting in damage to lipids, proteins and DNA in a variety of cells.[61,62]

Specifically, it has been shown recently that oral glucose challenge stimulates reactive oxygen species, or ROS, generation by blood mononuclear cells.[63] As has been hypothesized by us, the individual (that is on the person-to-person basis) shift in the ratio between hormonal (blood insulin) and genotoxic (ROS generation in mononuclears by luminol-dependent/latex-induced chemiluminescence) effects of glycemic load may reflect a Janus, or dual, role of glucose and probably can be associated with predisposition to the certain type of human pathology.[9,10,57] It was assumed that even among healthy people different reaction to glucose can be apparent. Preferential inclination of the probands to endocrine or genotoxic predominance may occur and this working hypothesis was confirmed by subsequent research.[10,11] Thirty eight healthy subjects (37 females, 1 male, age 19-58 years) without signs of glucose tolerance impairment were included into the study. All participants were given glucose (40 g/1 m^2 of body surface) after a 12- to 14-hrs overnight fast. Venous blood samples were taken at 0 and 120 min and processed for the preparation of mononuclear cells and for hormonal-biochemical measurements.

The average stimulation of ROS generation in mononuclears (parameter A) by oral glucose was equal at 120 min 1,77 (or + 77%) in the entire studied group. When ROS stimulation (120/0 min) with factor ≥2 had been evaluated it was observed in 9 of 38 subjects (i.e., in 23,7%). This group of people was designated as "GIGT+", or the group with glucose-induced genotoxicity. Correspondingly, the second group of 29 people (in which this stimulation was less than 2 or was not discovered at all) was designated as "GIGT−".[11] When several additional parameters have been compared in these groups, it was revealed that along with relative predominance of glucose-stimulated ROS generation over the level of reactive insulinemia (see parameter A/B), the only other noticeable distinction seen in people who belonged to the group "GIGT+" was lower glucose-induced C-peptide secretion (Table 2). This shows that "GIGT+ individuals" are really characterized by combination of increased glucose-induced ROS production and lower β-cells reaction to glucose (notably, the absence of distinctions in absolute values of insulinemia may reflect not only process of insulin production but also the rate of its biological clearance).[58] No difference between two compared groups was discovered in relation to the age of subjects, their BMI value, or levels of reactive glycemia, basal lipidemia and concentrations of thiobarbiturate-reactive products and carbonylated proteins (Table 2). Yet, a rather clear tendency to higher plasma levels of the TNF-α and lower concentrations of blood leptin (especially at 120 min) was observed in "GIGT+" group of subjects (Fig. 3). Although it is well known that increased TNF serum content may be associated with insulin resistance,[50,51] glucotoxins of different origin including alpha-dicarbonyl methylglyoxal are considered as an inductors of TNF.[64] Therefore, the combination of TNF excess with glucose-induced genotoxicity seems rather possible, perhaps reflecting a link in the chain of further pathological reactions. Additionally, the observed increase in the

Table 2. Comparison of the data in groups with and without glucose-induced genotoxicity, GIGT

	Group		
Parameter	**GIGT–**	**GIGT+**	**p**
Age	38,1 ± 2,3	34,9 ± 3,4	0,50
BMI	26,1 ± 0,8	25,6 ± 2,1	0,81
Glucose, 120/0 min	1,012 ± 0,037	0,922 ± 0,059	0,23
CML, 120/0 min (A)	1,05 ± 0,09	4,09 ± 0,73	0,0002
Insulin, 120/0 min (B)	11,96 ± 5,63	16,51 ± 13,2	0,71
A/B	0,43 ± 0,11	2,78 ± 0,93	0,0004
C-peptide, 120/0 min	3,84 ± 0,72	2,01 ± 0,59	0,19
CHOL, mmol/l	5,78 ± 0,21	5,71 ± 0,43	0,86
TG, mmol/l	1,01 ± 0,07	0,91 ± 0,07	0,48
LPS, cond.units	335,4 ± 20,0	323,2 ± 27,8	0,75
TBRPs, nmol/l	3,37 ± 0,29	3,90 ± 0,70	0,41
CP, cond.units	313,9 ± 24,9	294,3 ± 39,8	0,68

BMI—body mass index; CML, 120/0 (chemiluminescence data in mononuclears, cond. un., on 120 min. after peroral glucose load, to chemiluminescence data in blood mononuclears isolated after fasting); Insulin, 120/0 (ratio of blood insulin level on 120 min. after peroral glucose load, to fasting insulinemia); CHOL—blood cholesterol; TG—triglycerides; LPS—total (β + preβ) lipoproteins; TBRPs—thiobarbiturate-reactive products; CP–carbonylated proteins. Reprinted from Berstein LM, Vasilyev DA, Poroshina TE et al. Hormone Metabol Research 2006; 38:650-5 with permission from Georg Thieme Verlag KG.

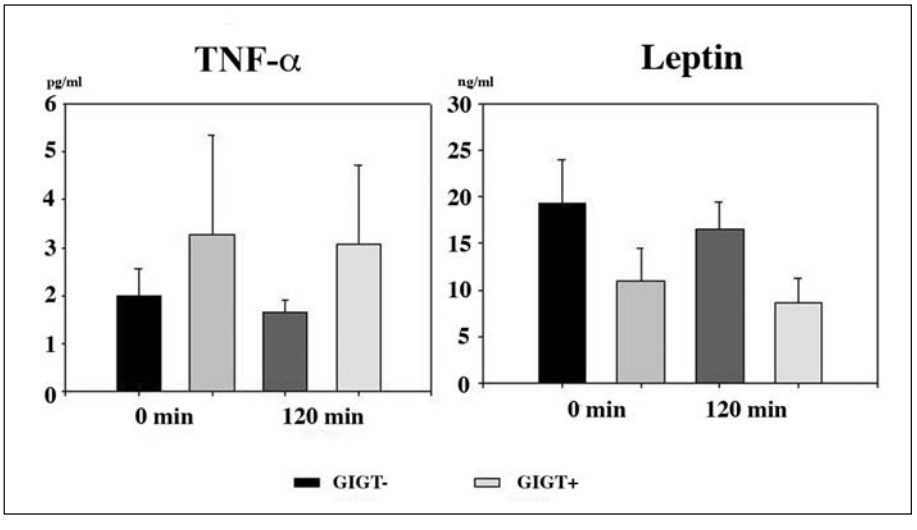

Figure 3. Plasma TNF-α and leptin concentrations after fasting and on 120 min of oral glucose load in subjects without (GIGT–) and with (GIGT+) signs of glucose-induced genotoxicity.

ratio of TNF-α to leptin seems rather characteristic and will be discussed below in the section entitled "Adipogenotoxicosis".

According to the data of S.W. Choi et al[65] the higher was the basal glycemia level in diabetic patients, the higher was rate of DNA damage in their lymphocytes (COMET assay). In our studies, notwithstanding the absence of difference between compared groups in glycemia level (Table 2) and in the comets' tail length **in the basal state, a tendency to higher stimulation of comet processing** with H_2O_2 was discovered in "GIGT+ subjects". Thus in aggregate, if dual function of glucose is realized in the "genotoxic mode", the phenotype (and probably genotype) of probands may be rather distinctive to that discovered in glucose-induced "endocrine prevalence". As a result, a specific pro-endocrine or promutagenic basis for different chronic diseases or for different features of the same disease can be created.[9-11] Such an assumption, in addition to other evidence, is supported by the data indicating signs of DNA damage only in subgroups of patients with atherosclerosis as well diabetes.[60,66] When searching for the factors which may promote a switch into the direction of glucose-induced genotoxic effect it should be underlined that though aging and obesity are sometimes considered as an inductors of excessive ROS production due to the insulin-related reactions, there are also data which (depending of cellular context) contradict with such notion.[67] Of note, our preliminary data show that tumor presence appears to be more important than the age of subjects in securing of the mentioned switch.[68] Of course, other possible modifiers of dual (Janus) function of glucose should be taken into account too. For instance, smoking can influence glucose tolerance starting from young adulthood[69] and according to our observations the incidence of smokers in "GIGT+" group was higher than in "GIGT–" subjects. Altogether, mechanistic and clinical associations related to presented findings as well as their significance for preventive measures deserve attention and further exploration.

Adipogenotoxicosis

Under conditions of glucose intolerance, free fatty acids acquire functionality as the principal energetic substrates, in accordance with the Randle cycle. Their excessive oxidation together with an age-dependent decrease in mitochondrial function, dysfunction of receptors of peroxisome proliferator-activated receptors (in particular PPARγ), etc., assist in furthering the progression of insulin resistance and the formation of a cluster of other metabolic propathogenic factors.[49,50,70] These observations together with data on the association of obesity with insulin resistance and increased cancer risk[50,71,72] (see also chapter of A. Hjartaker et al. in this volume) quite naturally rekindle interest in the role of adipose tissue. The latter, viewed previously as primarily an energy depository, is actually a functionally active endocrine organ producing steroid hormones (including estrogens) as well as hormone-like peptide molecules known as adipokines or adipocytokines (leptin, resistin, adiponectin, PAI-1, TNFα, visfatin, etc.).[73,74] Peptide hormones of adipose tissue may influence tumor growth directly as well as through the reproductive system and other mechanisms[75] and their problastomogenic effects may differ considerably. For example, leptin (probably via activation of MAP-kinase) increases aromatase activity and the proliferation index in mammary cancer cell lines, while a decrease of blood adiponectin concentration is described as a prospective risk factor for breast and endometrial cancer.[76,77] Accordingly, special attention is directed to mammary fat, since it is essential for the development of mammary epithelium. This occurs by providing signals that mediate ductal morphogenesis, by playing a vital role in defining the level of stromal-epithelial interactions and by contributing significantly to tumor growth starting from its early stages until further distinct clinical progression.[79,80] It is appropriate to re-emphasize here again that mechanisms of hormonal carcinogenesis, besides stimulation of cell proliferation, include formation of DNA adducts and mutagenesis in the target tissue.[6-8]

Importantly, adipose tissue consists not only of adipocytes but also of several other cell types, including macrophages.[73,74] Macrophages as a part on the nonfat compartment of adipose tissue, are increased in obesity and as a result of certain hormonal and nonhormonal signals.[81,82] They are responsible for almost all adipose tissue TNFα expression and for significant amounts of nitric oxide (NO) and IL-6 production.[73,81] These products are often considered as pro-inflammatory

mediators and effectors resulting in oxidative stress[83] and, finally, in the genotoxic cellular damage. Since features of oxidative stress were indeed reported recently in human adipose tissue and its cell lines,[84-86] we decided to study properties of mammary fat in breast cancer patients. Our aim was to find factors possibly contributing to the shift of these properties from hormonal to pro-inflammatory/genotoxic. We have coined the term, adipogenotoxicosis to characterize this process.[12]

Samples of mammary fat located 1.5-2.0 cm from the tumor have been taken within 10-15 min after surgery in 95 patients with breast cancer. The tumors were mainly intraductal breast carcinomas, stages $T_{1\text{-}2}N_{0\text{-}1}M_0$. Twenty five patients (mean age 42.6 ± 1.3) that had menses comprised the premenopausal group. The others 70 patients (mean age 63.2 ± 1.0) were postmenopausal for not less than 1 year. Among the latter, 23 patients showed signs of modest fasting hyperglycemia (6.1-7.5 mmol/l, n = 15) or overt compensated diabetes mellitus (n = 8). With respect to body mass most patients (>70%) were normal (BMI 18.5-24.9) or overweight (BMI 25.0-29.9) but not obese (BMI >30.0). Correspondingly, taking all this into account we compared the ability of mammary fat explants from premenopausal or postmenopausal breast cancer patients to release substances associated with adipocytes (leptin, adiponectin) or non-adipose cells, mainly macrophages (TNFα, IL-6, NO), into culture medium. In addition we studied the release of thiobarbituric-reactive products (TBRPs), a marker of lipid peroxidation, as well as aromatase activity and estrogen 4-hydroxylase (CYP1B1) expression in mammary fat.[12]

The most demonstrative results are presented in Table 3. Immunohistochemical staining for macrophage marker CD68 did not differ between the two groups. However, the release of NO and, especially, TNFα from adipose tissue showed a tendency to increase in the postmenopausal period (oppositely to the trend demonstrated by leptin and adiponectin). This was manifested also as a quite notable tendency to increase in the TNFα/adiponectin ratio from 4.56 ± 1.32 in the premenopausal group to 8.60 ± 2.06 in the postmenopausal group.

The menopausal status (pre or post) of cancer patients did not affect aromatase activity in their mammary fat samples, contrary to CYP1B1 expression and TBRP release into culture medium, which were higher in the premenopausal group (Table 3). In the latter group, in case of higher CYP1B1 expression and NO and IL-6 release, an increased aromatase activity in adipose tissue was found. In postmenopausal patients with fasting hyperglycemia, IL-6 level and IL-6/adiponectin ratio in incubation medium were notably higher than in the patients with normal blood glucose (Fig. 4). Thus, trends in the ratio TNFα/leptin in "GIGT+" group (see previous section), in mentioned several lines above ratio TNFα/adiponectin in mammary fat of postmenopausal patients and in the ratio IL-6/adiponectin under influence of hyperglycemia (Fig. 4) allow to conclude that just these parameters demonstrate in certain situations the domination of inflammatory/progenotoxic signs with rather high constancy. Notably, no differences were found between breast cancer patients with body mass index above or below the average as concerns the secretion of TNF, IL-6 and NO, as well as adiponectin and leptin by adipose tissue. This demonstrates, that unlike the role of menstrual status and glucose intolerance, obesity was not a factor promoting the shift from hormonal to genotoxic properties of adipose tissue in our studies.[12]

Thus, attributing to the features of progenotoxic switch in mammary fat not only an upsurge of TNFα, IL-6 and NO (related mainly to nonfat cells[73,81]) but increased expression of CYP1B1 as well and taking into account discovered aromatase-related 'associations', it may be concluded that this switch, or adipogenotoxicosis, is present not only in the postmenopausal (elderly) breast cancer patients. Besides, estrogens and their catechol derivatives are likely to be implicated in it to a not lesser extent than the well-known aforementioned pro-inflammatory molecules are. A tendency for the simultaneous decrease in the local release of peptide hormones (primarily, adiponectin) derived from adipocytes suggests that adipogenotoxicosis combines the loss of certain endocrine functions and the gain of progenotoxic effects. The mediating role of free fatty acids as ROS inductors[87] and recent data on TNFα ability to cause DNA damage through the generation of ROS[88] deserve mentioning too.

Future studies of adipogenotoxicosis should be focused on correlations between the hormonal and progenotoxic properties of adipose tissue and the clinical and morphological characteristics of

Table 3. The release of studied factors to the medium, aromatase activity and expression of CYP1B1 and CD68 in mammary adipose tissue of breast cancer patients

Group of Patients	Leptin (ng/100 mg of Tissue)	Adiponectin (mcg/100 mg of Tissue)	TNFα (pg/100 mg of Tissue)	NO (ng/100 mg of Tissue)	TBRPs (Extinction Units/100 mg of Tissue)	Aromatase Activity (fM/mg Protein/hr)	CYP1B1 (Conditional Units)	CD68 (Conditional Units)
PreMP	38,0 ± 9,9	8,4 ± 0,4	49,3 ± 9,5	283,6 ± 46,9	3,95 ± 0,63	7,22 ± 1,23	2,73 ± 0,18	2,47 ± 0,27
PostMP	25,5 ± 5,7	7,7 ± 0,2	83,1 ± 10,7	389,9 ± 52,8	2,54 ± 0,23	7,07 ± 0,75	2,25 ± 0,16	2,17 ± 0,17
p	0,24	0,13	0,06	0,27	0,01	0,92	0,08	0,35

PreMP—premenopausal; PostMP—postmenopausal. Methods: leptin, adiponectin, TNFα by ELISA, NO (nitric oxide) by Griess reaction, TBRPs by reaction with thiobarbituric acid, aromatase activity as 3H2O release from 3H-1-β-androstenedione, CYP1B1 and CD68 by semi-quantitative immunohistochemical evaluation. Reprinted from Berstein LM, Kovalevskij AY, Poroshina TE et al. International J Cancer 2007 with permission from John Wiley & Sons Inc.

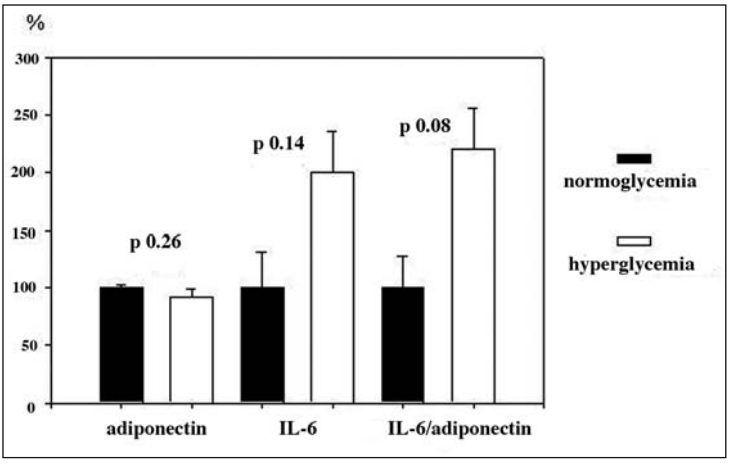

Figure 4. Release of adiponectin and IL-6 by mammary fat explants of patients with normo- and hyperglycemia.

breast cancer in order to check whether genotoxic shift is associated with a less favorable course of the disease. These studies should take into account the comparison of fat located in close proximity to a tumor and distantly from it (with the aim to address "the cause and effect" problem). If the adipogenotoxicosis hypothesis is confirmed, this may lead to development of specific fat-targeted interventions with the intent of preventing and treating cancer and probably some other main chronic diseases.

Basic Triad: Interactions and Implications

Figure 5 provides a brief overview of the issues addressed in this treatise. The triple endocrine-genotoxic switchings in estrogen, glucose and adipose tissue 'systems' composing the so called 'basic triad' can occur independently as well as interact with each other. Examples of such interactions include a trend toward adipogenotoxicosis in subjects with glucose intolerance (Fig. 4) and an association of PPAR-γ and -α and their target gene UDP-glucuronosyltransferase 1A9 simultaneously with glucose utilization, sensitization to insulin, free fatty acids mobilization and inactivation of genotoxic catecholestrogens.[89] Tobacco smoking appears to be a rather universal inducer of the three phenomena (PSEE, Janus/dual function of glucose and adipogenotoxicosis). Of note, prolonged smoking is able to increase the rate of many noncommunicable diseases (NCD). Furthermore, when the incidence does not increase, as in the case of breast and especially endometrial cancer,[90] the course of such diseases in smokers is characterized with poorer clinical outcomes.[40]

Even though human aging does not have a specifically ordered, biologically based program,[55,91] its type is no doubt of importance for the most people. The idea that hormone-related genotoxic shifts are associated with higher NCD aggressiveness and "less successful aging" is supported by several sets of observational data including: the apparently decreased survival in breast cancer patients with higher concentration of certain catecholestrogen fractions in tumor tissue;[92] the more frequent and severe diabetes complications in patients with the signs of oxidative stress and DNA damage;[60,61,93] and the correlation of DNA adducts in cells of thoracic aortas with stage of atherosclerosis.[66,94] Since fetal programming is considered nowadays rather frequently as a starting point for the rise of human pathology characteristic for the second half of life and even as a cause of reduced longevity,[95] attempts were made to find deviations in DNA adducts in young healthy adults born with low birthweight in comparison with age matched normal birthweight controls.[96] Of note, so called 'edge effects' of hormones on the very early and late stages of ontogenesis may involve a DNA-destroying mechanism appearing as a characteristic feature of their procarcinogenic

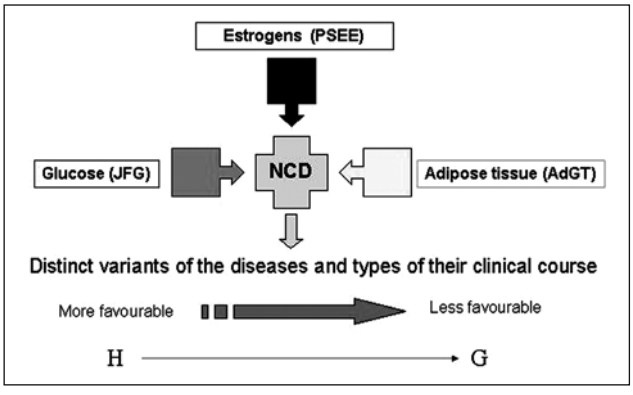

Figure 5. The components of 'basic triad' or triple endocrine-genotoxic switchings: possible association with noncommunicable diseases (NCD). PSEE—phenomenon of switching of estrogen effects; JFG—Janus/dual function of glucose; AdGT—adipogenotoxicosis; H, G—transition from hormonal effects to predominance of genotoxic effects.

influence.[9] Thus, all three mentioned allied or independent events (adipogenotoxicosis, Janus role of glucose and PSEE) should be viewed when discussing mechanisms of the development of major noncommunicable human diseases. The potential existence of two types of aberration with endocrine or genotoxic predominance should be considered and related to measures for their prevention. The aims of prevention should include well-known targets but also try to go beyond them. In this regard an advisable approach might include: correctors of steroid and peptidergic signaling (SERMs, SARMs, modifiers of aromatase, IGF-1, Ras-MAPK-PI3-kinase-system and so on—see chapters of R. Santen et al.; S. Bulun and E. Simpson; S. Sengupta and V.C. Jordan; T. Powles); alleviators of glucose intolerance and insulin resistance (e.g., diet and dietary restriction, biguanides, statins, glitazones, cannabinoid receptor blockers),[49,55,97-100] various antioxidants and antigenotoxicants,[21,101,102] more or less selective mTOR inhibitors,[55,103,104] but also an effort to reach the optimal balance in the ratio of the hormonal and genotoxic effects discussed above.

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