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PSYCHOSOCIAL (EMOTIONAL) STRESS. STEROID HORMONES AND THE GENESIS OF CANCER-MOLECULAR ASPECTS

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1.0. INTRODUCTION

Cancer is a disease of multifactorial etiology. Experimental studies on animals and epidemiological studies on human carcinoma indicate that a range of chemical, physical and viral environmental factors are involved in carcinogenesis and that several mutagenic events are necessary to transform a normal cell into a malignant cell[1]. *Development of tumors in humans and experimental animals proceeds through an extremely complex multi-step process, consisting of at least several stages, called «initiation», «promotion» and «progression». There is also a high correlation between cancer and traumatic psychosocial events. Psychosocial and emotional stresses play an important role in carcinogenesis acting as endogenous carcinogens*[2].

Recent experimental, psychological and epidemiological data indicate that the development of some forms of cancer can be related to certain cognitive, emotional and behavioural characteristics such as helplessness, depression, denial and inability to express one's feelings and needs, lack of aggression and high degree of social conformity[3].

It is well established that the emotional stress causes neuroendocrine

hormonal and metabolic imbalances, leading to hypersecretion of steroid hormones and increased levels of circulating glucocorticoid and gonadal steroids. Generally this increases cancer incidence in humans and animals. The actions of over-secreted steroid hormones may be neuroendocrine, paracrine, and autocrine. Through neuroendocrine and paracrine pathways, these steroid hormones may induce and/or maintain malignancy through autocrine action, although their paracrine and endocrine activities could also be sometimes implicated in the development of the tumors. Stress induced excess of steroid hormones may cause cancer by: (1) activating cellular or proviral oncogenes; (2) overproducing carcinogenic epoxides from dietary precursors and/or surplus circulating cholesterol and steroids-through increased activity of steroid hormone - induced microsomal enzymes; (3) impairing the immunological surveillance of the latent, malignant cells. (4) The action of steroid hormones is mediated by receptor-hormone complexes, and plays an important role in carcinogenesis[4]*. In this lecture I will attempt to summarize available data on the role of stress induced steroid hormones oversecretion and their receptors, in carcinogenesis.*

Data I will attempt to present to you resulted from a fruitful cooperation between Dr Ronald Grossarth-Maticek-Heidelberg and his group and our Institute of Molecular Biology and Endocrinology at the Vinča Scientific Centre (similar to Greek Demokritos Centre). Our duty was to translate psychological effects to molecular mechanisms underlaying stress-induced carcinogenesis. Consequently we proposed a highly hypothetic model whose postulates are under examination. In this lecture I will elaborate some postulates of the proposed model.

2.0. MECHANISMS OF CARCINOGENESIS

Many different cellular genes and molecular mechanisms can convert a normal cell through its progeny into malignant tumor cells.

Obviously, chemical carcinogenesis, unlike viruses, cannot introduce new genetic information into the genome of target cells. They must therefore act by covalently interacting with DNA and / or DNA-binding regulatory proteins to alter the structure, function and expression of cellular «proto-oncogenes» and thereby initiate malignant transformation[5]*. Chemical carcinogens may induce cancer in various ways, such as: (1) point mutation or rearrangements within promoter and/or enhancer sequences; (2) proto-oncogene mutation or*

gene rearrangements[6]*; (3) protooncogene amplification*[7]*; (4)gene translocation;(5) gene modification by phosphorylation and acetylation of chromosomal proteins (high mobility group protein HMG-14 and HMG-17)*[8]*; (6) gene methylation, DNA cytosine methylation, and demethylation (activated genes are demethylated)*[9]*; (7) promote the insertion of a strong viral LTR enhancer which triggers the spontaneous expression of cellular proto-oncogenes* [5,6,10].

Viruses transform cells either directly through a viral oncogene or, if they lack an oncogene, by inserting their promoter sequences near or within proto-oncogenes that would activate or enhance proto-oncogene expression[5,6]*. Insertion of viral DNA into the cell genome is by itself potentially mutagenic: it can directly damage cellular regulatory or structural genes and alter their expression by bringing them under control of strong viral LTR enhancer/promoter elements*[4-6]*. Such «insertional mutagenesis» has been implicated in tumorogenesis by a variety for retroviruses.*

There are more than 100 proto-oncogenes and oncogenes[6]*. Yet, at present we know only four biochemical mechanisms by which this rich diversity of oncogene proteins may act: protein phosphorylation by tyrosine — or serine — and threonine-specific protein kinases*[11]*; metabolic regulation by proteins that bind GTP like the familiar G proteins*[12]*; control of gene expression,* [13-15]*: and the control of the initiation of DNA replication by oncogene products, such as myc and fos genes products*[16].

Steroid hormones, oversecreted due either to neuroendocrine disorders or malignancy, may affect all of the carcinogenic mechanisms or maintain the malignant state of the cell. They could regulate oncogene expression[4-6]*, the functioning of oncogene products*[6]*, and protein phosphorylation*[3,4,6,11]*, because steroid receptors or their subunits seem to have kinase activity. They also affect the activity of the microsomal enzymes that transform procarcinogens into active carcinogens (mutagens)*[3,17]*, and they could alter the immune competence of the organism*[3]*. These are the reasons why steroids and their receptors have become important in contemporary molecular and genetic oncology.*

3.0. MECHANISM OF STEROID HORMONE ACTION

A central problem in the molecular biology of today is the understanding of the mechanism by which hormones (chemical signals) regulate, in a temporal and tissue-specific manner, the expression of specific target genes. The re-

sponse to steroids is strictly tissue specific, but a single gene in a particular tissue could be regulated by several steroids-peptide hormones, neurotransmitters, and oncogene products. The steroid-responsive elements in the promoters of target genes have enhancer-like properties. Up to now, the best characterized steroid-responsive elements are the glucocorticold responsive elements (GRE) of the mouse mammary tumor virus (MMTV)[19]*. They are situated upstream or downstream from the transcription initiation site. Steroid-responsive enhancers also have been identified in long terminal repeat (LTR) sequences of the Moloney mouse sarcoma virus and coupled to several cellular genes*[20,21]*. In addition to the interaction of the steroid hormone-receptor complexes with the hormone-responsive elements of their target genes and transcription regulatory factors, other regulatory proteins play an important role in transcription*[22]*. Steroid receptor proteins belong to the family of transcription regulator proteins*[21,22]*.*

It is evident from the available data that the specific steroid receptor complex is a transcription regulator which activates and regulates target gene expression by interacting with specific regulatory elements. It may activate or suppress the transcription (expression) of oncogenes. Consequently, any change in the structure of the steroid receptor may influence the specificity and the rate of gene expression as well as carcinogenesis. That is why we paid particular attention to the role of steroid receptors in normal target cells and in malignant cells.

3.1. EMOTIONAL STRESS AND CARCINOGENESIS-EXPERIMENTAL DATA

Although the molecular mechanism(s) of psychosocial stress is only very poorly understood, very scarce animal experiments have shown that high levels of stress (e.g., isolation of mice) can both increase the incidence of cancer and promote the growth of cancer[23,24]*. The appearance of spontaneous tumors is usually earlier among the stressed than the non-stressed subjects*[25]*. At present there is no exact answer to the question of how psychosocial stresses may influence the carcinogenesis. It is shown that anxiety stress produces increased levels of steroid hormones as well as pituitary hormones, cathecholamines and neurotransmitters through the well known neuroendorcrine axis involving cerbral cortex, hypothalamus, pituitary, adrenal and gonadal glands (HPA - axis). It should be noticed that the enhancement of neoplastic processes and the escape from host immunologic control can be demonstrated in mice and*

other animals by either the induction of anxiety or by injection of corticoids at stressed levels. It has also been demonstrated that the mouse mammary tumor virus (MMTV) gene expression is regulated at the transcriptional level by glucocorticoid hormone receptor complexes (ref). The sequences within the MMTV long terminal repeat (LTR) are involved in the hormone regulation. Glucocorticoid receptor complex binds preferentially to LTR DNA fragments (ref) and LTR confers glucocorticoid regulation on linked heterologous genes[26]. *The androgens, via specific receptors, are also involved in the regulation of viral sequences in mouse mammary tumor cells*[27]. *Mammary tumor cells (S115) carrying the MMTV sequences are expressed in response to androgens. Sites near or within the LTR of S115 DNA seemed to be more extensively methylated in the androgen unresponsive (A^- cells) than in androgen responsive (A^+ cells). However DNA methylation seems to be implicated in the control of MMTV expression*[28], *and in tumorogenesis in mice*[29], *but the evidence of its overall role in eukakryotic gene expression is conflicting*[30]. *Binding of the hormone-receptor complex to DNA LTR sequences induces (1) a local change in chromatin conformation or (2) it provides a target or entry site for RNA-polymerase or for its accessory factors.*

3.2. STEROIDS AND CARCINOGENESIS

The role of hormones in the process of carcinogenesis has been the subject of controversy, but there is a lot of evidence for naturally occurring steroid hormones and their synthetic analogues being involved in tumor initiation and/or progression[31], *as well as in tumor inhibition*[32]. *Steroid hormones have been implicated in abnormal growth regulation both in tumors and tumor-derived cell lines*[33,34]. *The growth of hormone-dependent mammary cancers is thought to be stimulated mainly by estrogen and prolactin, while androgens, progesterone, glucocorticoids, and synthetic antiestrogens have been reported to reduce tumor growth*[35]. *There are reports suggesting that excess doses of estrogens are connected with the human ovarian tumor*[36] *as well as with the endometrial adenocarcinoma*[37]. *Dependence of prostate growth and function on circulating androgens is well established. Benign hypertrophic and cancerous prostates have more androgen-receptor than normal tissues*[38].

It is well documented that estrogens also affect neoplasia in non-genital organs, such as the kidney, liver, lymphoid tissue, meninges, salivary glands, skin, and urinary bladder[39–41]. *A lot of data exist showing that renal carcino-*

mas have been induced by estrogens in experimental animals[42]. *The incidence of human renal carcinoma is more common in males, and it is proposed that progesterone secretion protects women from this tumor*[41]. *Antihormones or hormone antagonists have been used to slow down or block tumor growth and prevent metastases or to induce their regression following nephrectomy. Human liver from both sexes contains estrogen receptors*[43]. *Of particular interest is the role that estrogen and its receptors may play in pathogenesis of hepatic neoplasia. There is evidence relating the use of oral contraceptive steroids to the development of both benign and malignant hepatic tumors*[44,45], *focal nodular hyperplasia*[46], *and hepatocellular carcinoma*[47]. *When estrogen receptor levels were estimated in normal and cancerous tissues, it was revealed that the hepatic adenoma contains significantly fewer cytosolic estrogen receptors, but significantly more nuclear estrogen receptors than normal liver tissue. Hepatic adenoma seems to be more responsive to estrogenic hormones*[48]. *It has recently been shown that the possible mechanism by which estrogen induces kidney cancer is induced by endogenous DNA adduction, that is, estrogen induces the binding of some unknown endogenous compound(s) to DNA. It has been postulated that this mechanism plays a key role in hormone-induced malignancy*[49].

There are some indications that glucocorticoid hormones are involved in tumor growth[50]. *It was shown that medroxyprogesterone acetate is one of the most efficient inhibitors of tritiated dexamethasone binding, suggesting that this widely-used compound for treating metastatic renal cancer may cause tumor regression by binding to the glucocorticoid receptor, thereby eliminating the growth-promoting action of endogenous glucocorticoids*[51].

It is difficult to speculate on the exact pathway by which glucocorticoids can influence kidney carcinogenesis because they have a wide range of effects on the kidney cell. It was suggested that this hormone could regulate kidney growth by inhibiting ornithine decarboxylase[52], *or by inhibiting the expression of the plasminogen activator gene and the enzyme's activity, which play an important role in many aspects of cellular regulation including tumor metastases*[53]. *Alternatively, the glucocorticoid might act by activating a latent oncogenic virus, because it has recently been shown that steroid responsive MMTV proviruses are integrated in the DNA of kidney adenocarcinoma cells. A glucocorticoid-enhanced MMTV production might increase the chances of viral integration near, and activation of, a putative proto-oncogene*[54]. *The*

presence of oncogenic viruses has been reported in different renal tumors[41]. *Although the mechanism(s) by which steroids become involved in malignant transformation has not been elucidated yet, certain links exist, though very complex and not well understood. Steroids regulate the wide range of physiological processes such as metabolism, differentiation, and growth by regulating the transcription of specific genes in the target cells*[55]. *Steroid hormones exert their biological action through intracellular receptors, which belong to the family of transcription factors. As to the possible role of transcription factors in malignancy, «altered» factors could play key roles in oncogenic transformation by altering the expression of target genes*[56–59]. *Furthermore, the mechanism of action of steroid hormones at both receptor and postreceptor levels may be closely related to the cellular mechanism of action of oncogene protein products. In addition to that, some oncogene products having protein kinase activity may regulate the activity of the steroid hormone-receptor complexes through phosphorylation/dephosphorylation processes. Also, the steroids could regulate transcriptional or post-transcriptional stages of the expression of some proto-oncogenes*[60]. *In addition to that, since some oncogene products and steroid receptors are members of the enhancer-binding protein superfamily*[57], *carcinogenesis could be the result of alteration of enhancer-binding proteins and/or enhancer elements of the key cellular genes*[58]. *The current challenge is therefore to understand how specific protein-DNA interactions regulate gene expression. Thus, the study of the steroid receptor structure, in normal as well as in malignant cells, may be important for better understanding of the role of steroid hormones in the regulation of gene expression as well as in malignant transformation.*

The oversecretion of steroid hormones, caused either by neuroendocrine - hormonal and metabolic disorders or by abnormal ectopic secretion, increases the circulating levels of adrenal and gonadal steroids. Both levels of steroid regulate gene expression, induce enzyme activity, control metabolic pathways, and regulate immunological competence[3,19–22]. *Elevated circulating levels of steroid hormones may provoke abnormal gene expresion and activate cellular or viral oncogenes*[3,4,6], *change patterns of signal transduction and enhance the endogenous production of chemical carcinogens by activating microsomal enzymes, and lower the immune capacity*[3,17]. *Thus steroid hormones, oversecreted due either to primary neuroendocrine disorders or to reprogrammed*

abnormal ectopic hormone secretion, may be involved in induction, promotion and maintenance of malignancy.

4.0. STEROIDS AND ONCOGENES

The recent cloning of glucocorticoid, estrogen and progesterone receptors and comparison of their amino acid sequences revealed extensive homologies among them[58]*. Human steroid receptors and the v-erb*A *gene product of avian erythroblastosis virus (AEV) are strikingly homologous and thus can all be considered as members of a superfamily of enhancer-binding proteins*[57,58,61–63]*. Also, c-erb*A *amino acid sequence, the cellular counterpart of v-erb*A*, is homologous to steroid receptors*[58]*. Comparison of the amino acid sequences of the viral v-erb*A *and human c-erbA protein products with the human glucocorticoid receptor indicates varying levels of homology with the carboxyterminal half of glucocorticoid receptor*[58]*. Although the transformation of the cell by AEV, which harbours the v-erbA gene and the v-erb*B *oncogene, is accomplished primarily through the activity of the v-erb*B *product, an epidermal growth factor (EGF) receptor without its EGF-binding domain*[64,66]*, the v-erb*A *gene product, a non-oncogene cytoplasmic P75 gag-erb*A *fusion protein, seems to potentiate transformation. Bearing in mind the above-mentioned homology between v-erb*A *protein products and steroid receptors, a question can be posed: could steroid receptors potentiate transformation like the p 75 gag-erb*A *protein? Furthermore, significant homology has also been found between the erb*A *product and the hormone-binding domain of region E of both glucocorticoid and estrogen receptors. Therefore, it was proposed that c-erb*A *is the receptor for a steroid*[62]*. Taking it all together, a few possibilities can be considered. Steroids can be involved in carcinogenesis by binding to the v-erb*A *protein, and under certain circumstances, the structure of steroid receptors might be altered to have a similar role in the transformation processes as the v-erb*A *product. The interesting and important finding was that c-erb*A *product also specifically binds thyroid hormone. These data led to the conclusion that the genes for steroid receptors, as well as for some other hormone receptors, and erb*A *genes have evolved from a common ancestor*[57,62,63]*. It should be mentioned that EGF receptor concentration is significantly higher in some carcinoma cells and that there is an inverse relation between EGF and estrogen receptor expression. In cell lines of human breast cancer, the absence of estrogen receptor expression is associated with higher levels of functional EGF receptor protein and*

mRNA[67,68]. *Also, glucocorticoid reduces the secretion of EGF in human salivary gland adenocarcinoma cell line, and although the mechanism of growth inhibition by glucocorticoids is still unclear, it could be due to reduced EGF secretion*[69]. *Whether the same relationship exists between the steroids and v-erb*B *is not known.*

One of the protooncogenes most studied is c-myc, the amplification, rearrangement, and translocation of which repeatedly has been observed in various tumors including breast carcinomas. It has recently been shown that estradiol increases the accumulation of the c-myc mRNA. This effect occurs only in estrogen receptor-positive cells[70]. *In mammary tumors the expression of c-H-ras gene is under the control of ovarian hormones, so the levels of ras proteins are much higher in estrogen and progesterone receptor-positive tumors than in those which are receptor negative*[71]. *For example, glucocorticoid hormones can only transiently stimulate a v-mos or v-ras gene coupled to the steroid-responsive MMTV LTR enhancer/promoter (in 3T3 cells) because the v-mos and v-ras products can somehow inhibit the MMTV LTR*[72]. *Glucocorticoids can also regulate the expression of chronic transforming retroviruses. The stimulation of MMTV expression by several classes of steroid hormones, including glucocorticoids, androgens, progestins, and mineralocorticoids is well documented: the steroid responsiveness is due to a 202-nucleotide domain which precedes the RNA initiation site*[73–77]. *Glucocorticoid, progesterone, and androgen receptors are bound to the same two regions of MMTV LTR although the footprints they produce are not identical. Glucocorticoid administration also enhances the number of retroviral particles in Ehrlich ascites tumors of mice*[78], *and increases the transformation of normal rat and human cells by Kirsten murine sarcoma virus*[79].

*It has recently been reported that HBV integrates into the liver cell genome near a gene which is closely related to both the erb*B *gene and the sequences coding for the binding domains of the human estrogen and glucocorticoid receptor genes*[80]. *It has been suggested that this gene is inappropriately expressed as a consequence of HBV integration. Since the HBV insertion takes place a few nucleotides upstream from the beginning of the coding sequence for the receptor's DNA-binding domain, it is most probable that the inappropriate activation of that gene as a consequence of HBV integration may result in expression of a truncated, DNA-binding receptor protein which could participate directly in the subsequent cell transformation*[80].

Results from diverse studies suggest that steroid-induced or related cancer may result from: direct initiation (steroid-epoxides) of malignant transformation, alteration of the specific gene expression; activation and modulation of proto-oncogenes expression; modification of the metabolism (liver, breast, prostate, kidney) of aryl mono-oxygenases, and depression of immunologic surveillance[3-6,22].

5.0. GLUCOCORTICOID RECEPTOR

In our laboratory in an extensive study lasting more than 10 years we attempted to identify and characterize the structure of glucocorticoid receptor (GR) system, using various methods as described in our previous papers[81-86].

Sucrose-gradient centrifugation indicated that the glucocorticoid receptor complex has a 10.4-S value under hypotonic conditions and in the presence of Na-molybdate. After Na-molybdate removal and subsequent thermal activation, there was a transition of the 10.4-S form of the rat liver glucocorticoid-receptor complex into 3.7- to 4-S forms[86]. *This shift in sedimentation coefficient from higher (8 to 10 S) to lower (4S) values is accompanied by the activation of the receptor*[81,86-89].

According to the state of activation different forms of glucocorticoid receptor complex were separated, by DEAE-Sephadex A 50-minicolumn chromatography[81,83,90]. *Only one receptor form could be detected in unactivated cytosol. Two different activated receptor forms were separated following the activation: protein II (P II) and IB*[81,83,91]. *The glucocorticoid receptors from various tissues (heart, kidney cortex, kidney medulla, liver, muscle, thymus, and different brain structures: septum, hippocampus and hypothalamus*[81,83]*) had varying PII/IB ratio which strongly suggested that protein II and protein IB are separate subunits of GR*[81]. *The interaction between an antihormone, cortexolone, and the liver glucocorticoid receptor supports this view. The in vitro binding of cortexolone to the receptor causes immediate activation of the glucocorticoid receptor and triggers the dissociation of the IB and protein II subunits. Cortexolone binds only to IB subunits. In vivo experiments showed that cortexolone and promegestone also bind only to 3.5-S IB protein. This finding, and our previous data, indicate that antiglucocorticoids bind only to IB subunit of glucocorticoid receptor and that this binding site is different from the other glucocorticoid binding site. Our results are in agreement with those of Turnell et al.*[92] *who isolated a 3.5-S cortexolone complex from the rat thy-*

mocytes. The existence of two different binding sites on GR, one for glucocorticoids and their agonists and the other for antiglucocorticoids, has been suggested by several authors[92–95]. *The IB protein has also been purified by chromatography on a cortexolone-21-mesylate-Sepharose 4 Ba ffinity column*[96,97]. *The properties of the IB protein obtained by cortexolone affinity chromatography were the same as those previously described in our publications*[81,83,85,97]. *However, considerable evidence has been reported from other laboratories indicating the existence of two different glucocorticoidreceptors, protein II and IB*[98–102], *whose relative concentrations vary from tissue to tissue*[98–103]. *The IB receptor subunit is responsible for the production of phospholipase inhibitory proteins, induced by dexamethasone and phenytoin (25-diphenylhydantoin, or DPH), IB also mediates glucocorticoid stimulation of cation transport in the rat colonic epithelia, which contain only receptor IB*[103,104]. *The fact that DPH occupies the steroid-binding site of IB, but not of protein II, and that it is a selective agonist of IB but not of protein II, suggests that IB is a separate gene product from protein II, although extensive homology would be expected. This seems to be the case since it appears that protein II, which mediates tyrosine amino transferase (TAT) induction and growth inhibitory functions of glucocorticoid, is coded by a gene(s) on the mouse chromosome 18, whereas IB protein which regulates extragenomic effects is coded by a gene(s) on chromosome 17*[103–105]. *We therefore concluded that IB and protein II are different subunits of the glucocorticoid receptor. Our postulate is also supported by the finding that heart muscle contains the highest relative amount of steroid-binding subunit II and the lowest relative amount of steroid-binding subunit IB. The white muscle that atrophies from glucocorticoids had a negligible amount of binding subunit II and the highest content of binding subunit II and the highest content of binding subunit IB* [89].

The liver and thymus cytosol glucocorticoid receptors have been purified, in our laboratory, to homogenecity by sequential chromatography on phosphocellulose, DNA-cellulose, and DEAE-Sepharose (three-step purification procedure)[95,100]. *The activated GR preparation, obtained after the final purification, contains a 94-kDa glucocorticoid binding protein as the dominant component and a 72-kDa co-purifying protein of unknown function, and perhaps a 24-kDa component of unknown composition. The 94-kDa steroid-binding Gr subunit is phosphorylated in a number of tissues*[95,106–108]. *Phosphoryl-*

ation of GR might play some role in the regulation of GR functions, such as hormone binding and activation[81,107,108].

All of our results obtained by different methods suggest that inactive (untransformed) glucocorticoid receptors are a multimeric complex which, during activation, reduces in size and dissociates into subunits. Several laboratories have also shown that inactive (untransformed) 9-S receptor is an oligomeric complex, containing a 90-kDa phosphoprotein which does not bind steroid[109-112]. *This protein is immunologically identical with, and has the same peptide map as, the 90-kDa heat-shock protein*[112]. *When glucocorticoid receptors are activated (transformed) the 90-kDa protein dissociates from the receptor. The 90-kDa protein is associated with all steroid receptors*[109,111,112]. *There is evidence that low-molecular-weight RNA can also be covalently cross-linked to the glucocorticoid receptor*[113]. *The inactive 9 to 10 S steroid receptor complex thus contains specific steroid binding and other subunits or components. Their physiological relevance to receptor functions has not yet been elucidated.*

We concluded that the glucocorticoid receptor is either a heteromultimer or a complex of several different regulatory proteins, which may vary from tissue to tissue[81,84,95]. *Its activation results in a dissociation of subunits or the release of associated components. Some receptor components might trigger hormonal extragenomic responses, while other components modulate gene expression*[81,84,85].

5.1. CLONING OF STEROID RECEPTOR GENES

Characterization of steroid receptors was seriously hampered by their low abundance in target cells (less than 0.01%). However, during the past 5 years, most of the steroid receptors have been cloned, sequenced, and the extent of their homology established[114,115].

The steroid-binding protein subunits of glucocorticoid receptors from the rat, mouse and human sources have been cloned, sequenced, and expressed[114]. *Chicken progesterone receptor*[115] *and the human and Xenopus laevis estrogen receptor*[61] *have also been cloned. All of these receptor proteins are closely related. The highest degree of homology is observed in their DNA-binding domains, suggesting that diverse groups of regulatory proteins may employ a remarkably conserved mechanism for transcriptional control. The DNA-binding domain of steroid receptors contains a series of clustered cysteine residues, which probably coordinate 3-Zn atoms to form «3 Zn-fingers» with which the receptor*

protein binds to DNA[22,114–117]. *In addition, there is evidence that this «Zn-finger» domain is also necessary for the modulation of gene expression*[22].

The greatest variability was observed in the N-terminal region of the steroid receptors. There are also tremendous variations in the size of the N-terminal domain which seems to be essential for the full transcriptional activity of the receptor, because deleting various lengths of the N-terminal domain decreases transcriptional activity in rat and human GR receptors[118,119].

The greatest variability was observed in the N-terminal region of the steroid receptors. There are also tremendous variations in the size of the N-terminal domain of receptors which have an excess of acidic residues. This N-terminal domain seems to be essential for the full transcriptional activity of the receptor, because deleting various lengths of the N-terminal domain decreases transcriptional activity in rat and human GR receptors[118,119].

The most important observation for the role of steroid receptors in carcinogenesis is that deletion or frameshift mutations in the region of the genes coding for the steroid-binding domain may result in the production of «truncated» receptor which is a steroid-independent, spontaneously active transcription factor[120]. *The uncontrolled transcription of target genes due to the constitutive activity of such truncated steroid receptors might trigger or promote carcinogenesis*[4–6].

At present, it is not known how the steroid receptors interact with other transcription factors to stimulate transcription[22].

5.2. PHOSPHORYLATION AND STEROID RECEPTORS

Phosphorylation of the receptors for progesterone[121], *estrogen*[122], *and glucocorticoid*[127] *has been demonstrated. The physiological significance of receptor phosphorylation remains to be established. It might play some role in the regulation of hormone binding and receptor activation, as suggested by Schmidt and Litwack*[123]. *These authors also suggested that the steroid receptors may themselves have protein kinase activity. It has recently been reported that partially purified preparations of type A progesterone receptor contained a kinase activity which phosphorylated the receptor more effectively than did cAMP-dependent protein kinase. Partially purified preparations of type B receptor contained another kinase activity which could phosphorylate the type B receptor*[124]. *In both cases, the kinase activity could be separated from the receptor subunits by affinity chromatography*[125]. *Both the A and B forms*

of the progesterone receptor are also in vitro good substrates for the epidermal growth factor (EGF) receptor kinase[126]*. Phosphorylation of tyrosine residues, as well as phosphoserine or phosphothreonine residues, was detected.*

The protein kinase(s) involved in the phosphorylation of the glucocorticoid receptor has not been identified. Several authors have reported that purified glucocorticoid receptors, obtained by steroid affinity chromatography, have endogenous protein kinase activity[123,127–129]*. However, the purified GR protein, from mouse fibroblasts, and the 94-kDa GR protein from rat liver, do not have intrinsic kinase activity*[130,131]*.*

We have observed a rapid increase in the rat liver GR phosphorylation in vivo following intraperitoneal injection of glucocorticoids. This phosphorylation increased the binding capacity of GR protein and its translocation to the nuclei[81,84]*. The same results were obtained in the presence of ATP*[132]*.*

We attempted to find out whether kinase activity is present in the liver GR preparation and, if so, whether this activity is associated with or intrinsic to the 94-KDa subunit of GR. We have found that in both rat liver and thymus protein, kinase activity is associated with purified activated GR[106]*. Our results also showed that this kinase phosphorylates serine and threonine residues and has a broad specificity for several substrates such as 94-kDa glucocorticoid binding subunit, various histone fractions, protamine, and regulatory (R) subunit of cAMP-dependent kinase*[106]*. The fact that this kinase was detected in both anabolic (liver) and catabolic (thymus) target tissues suggests that the association might be of physiological relevance for both the extragenomic and genomic functions of GR. The kinase might be involved in extragenomic events like immediate autophosphorylation of GR upon hormone binding*[81,84,95]*, and in the phosphorylation of the rat liver ribosomal protein S6 and several other cytosolic proteins which we observed 5 min. after glucocorticoid administration in vivo*[84,95,133]*. This kinase might also be associated with the 94-kDa receptor and translocated with the receptor to nuclei where it is involved in the modulation of target gene expression. It might also phosphorylate transcription factors.*

At the moment, the biological significance of the protein kinase activity of steroid receptors is unknown, but it might be of crucial importance for steroid hormone action. Indeed, the phosphorylation of chromatin proteins could well be involved in the promotion of transcription of the steroid-responsive genes.

5.3. THE STRUCTURE OF STEROID RECEPTORS IN MALIGNANT TISSUES

There is a lot of data showing that many kinds of human carcinomas are hormone-dependent tumors and that treatments with antihormones or hormone antagonists slow down or block tumor growth and prevent metastases or induce tumor regression. It is generaly accepted that only receptor-positive cells should respond to hormone treatment. It is difficult to understand the exact role of steroid receptors in carcinogenesis.

The existence of malfunctioning receptors has been put forward to explain why up to 50% of ER-positive patients fail to respond to antiestrogen therapy. Recently, partial screening of the ER mRNA from 71 mammary tumors with eight constructed subclones revealed a subpopulation of tumor mRNA with an altered sequence coding for the B region of the ER. It was suggested that this receptor variant corresponds to a particular subclass of ER-positive tumor carrying a biologically significant missense mutation. Such a mutation could affect the half-life of the protein, its solubilization properties, or it could lead to an imperfect regulation of the ER gene. Alternatively, the variant gene may contain a frameshift or nonsense mutation that will interfere with the synthesis or markedly alter the structure of the receptor molecules (e.g., truncated receptor which may be active without estrogen)[134].

We investigated the ER, PR, and glucocorticoid receptor (GR) samples of morphologically unaltered (termed control) and malignant tissue from the same kidneys with adenocarcinoma or papillary carcinoma of the renal pelvis. The results showed that inactivated 8-S GR, which can be separated as a 0.4 MKCI peak on the ionexchange column and does not bind to DNA-cellulose, was detected in only a few of the control samples, while in the remaining control tissues, as well as in malignant tissues, only the activated (4-S DNA-cellulose protein binding form) or even smaller form was obtained, PR and ER complexes were detected as 4-S or lower sedimentation coefficients only. These results show that the structure of steroid receptors may often be altered even in non-malignant samples derived from malignant kidney, suggesting that the altered structure of steroid receptors may be involved in the process of tumorogenesis. Another possible explanation of our results may be that steroid receptor gene can mutate in the process of malignant transformation and, as a consequence, produce structurally and functionally modified receptor protein[135–137]. *In cancerous human prostates, the androgen receptor (AR) sediments at 8.5 S*[138], *while in rat prostatic carcinoma the AR sedimentation pro-*

file varied with the histological type of the tumors examined, ranging from 7.8 S in moderately differentiated adenocarcinoma to 4.5 – 5 S in tumors with extensive proliferation. However, there was no detectable AR in prostate fibrosarcoma, the most rapidly growing tumor. In the normal parts of the same prostate tissues, the 8.3-S ER was detected, but, again, not in fibrosarcoma[41].

Spontaneous steroid receptor mutants are not rare in mouse lymphoma cells or human lymphoblastic leukemia cells. These cells normally stop proliferating and lyse in response to glucocorticoids, but the receptor mutants do not. The vast majority of these resistant mutants had different kinds of defective receptors[139]. *It has been considered unlikely that the 40-kDa receptor is formed by cleavage of a 90-kDa form*[140]. *Differences between the properties of GR in CR and CS lines of transplantable hamster melanomas have been reported. CS tumors are sensitive to glucocorticoid while CR tumors are unaffected. In CS cells two GR of 7 and 13 S were found, while in CR cells only the 7-S receptor form was revealed*[141].

In human tumor (leukemic, peripheral blood) cells, changes in GR structure were detected. Inactivated and activated receptors were eluted from DEAE-cellulose only as single, low-salt peaks of 2 – 2.5 S proteins in contrast to the elution pattern of GR from normal cells in which inactivated GR had both low- and high-salt peaks of 3.5-S and 8.5-S proteins, respectively. Receptors from abnormal cells displayed minimal affinity for DNA-cellulose[142]. *It has recently been suggested that altered receptor molecules may act in a deregulatory manner and induce celullar proliferation. It was proposed that the aberrant receptor-like molecules can bind to DNA and give a proliferation signal even in the absence of steroid ligand*[143]. *It was concluded that the steroid ligand is not absolutely required for generating the conformation of the glucocorticoid receptor that allows interaction with the regulatory elements in the long terminal repeat region of MMTV*[144]. *The finding that steroid hormones are not essential for receptor-mediated gene activation suggests that truncated receptors could play some role in progression of certain malignancies*[22,119]. *Since steroid receptor genes and some oncogenes are highly homologous, an aberrant receptor molecule might escape regulation by steroids and start to behave as an oncogene product. Taking into account our data on steroid receptor structure in malignant kidney tumors, as well as all other tumors, it is more and more evident that altered hormone receptors may be important in*

oncogenic transformation because they affect the transcriptional regulation of crucial target genes.

6.0. SIGNAL TRANSDUCTION AND CARCINOGENESIS

The extracellular chemical signal molecules are perceived by cellular receptors which are often located on the cell surface. The occupancy of these receptors triggers a cascade of events in the cytoplasm and nucleus while altering the pattern of gene expressions. Various phosphoprotein kinases play important roles in those signal-transducing events. It is nowadays evident that viral oncogenes or activated cellular proto-oncogenes code for components of the signal-transducing mechanisms[5,6]*. Any aberration in signal transduction may disrupt normal growth, differentiation, and intercellular coordination.*

There is now evidence that the platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin, and certain lymphokines bind to cell-surface receptors which activate a tyrosine kinase domain in the cytoplasmic portion of their receptors[1–6,11]*. Another mechanism of signal transduction is the beta-adrenergic system in which the activated receptor stimulates the adenylate cyclase, through a G-protein mediator. The resulting increase in cytoplasmic cAMP activates the cyclic AMP-dependent protein kinases which phosphorylate serine and threonine residues*[145]*. At present, the role of this pathway in growth control is not clear. The adenylate cyclase signal transduction pathway can stimulate or inhibit growth and proliferation depending on conditions. Certain prostaglandins, neurotransmitters, and peptide hormones act via adenylate cyclase-coupled receptors*[4,81,146]*.*

The third pathway of signal transduction involves the activation of a family of phospholipid- and Ca^{2+} — dependent serine — and threonine-specific protein kinases and the protein kinase C, which plays an important role in a variety of membrane-related signal transduction events[147]*. The activation of a phospholipase C leads to hydrolysis of phosphoinositol 4,5-diphosphate to diacylglycerol and inositol 1,4,5-triphosphate. The diacylglycerol then activates the kinase C. Inositol triphosphate triggers the release of Ca^{2+} from ER-like Ca^{2+} -storage vesicles. The resulting cytoplasmic Ca^{2+} surge then activates several Ca^{2+} calmodulin-dependent enzymes (protein kinases, phosphatases, phosphodiesterases) and activates the cytoskeleton. The tumor promoter 12-0-tetradecanoylphorbol-13-acetate (TPA) and related tumor promoters, apparently act by increasing the activity of the protein kinase C.*

The specific substrates of the various kinases are not yet identified, but they include receptors and membrane-associated ion channels[145,147]. *A major gap in our knowledge is the mechanism by which signals are ultimately relayed to the nuclei and the mode of their action on different levels of the genome and gene expression.*

6.1. THE ROLE OF STEROID RECEPTORS IN SIGNAL TRANSDUCTION

Extragenomic events preceeding gene expression play an important role in signal transduction mechanism(s). Signal transduction involves a rapid cascade of events such as phosphorylation, methylations, and acetylations of preformed regulatory proteins[8,9]. *There is a large family of genes whose products are transcriptional regulatory proteins*[22]. *The steroid hormone receptors are a family of regulatory proteins whose ability to control gene activity (expression) depends on their activation by their steroid ligands. Steroid receptors are phosphoproteins*[106,127,149]. *They are also substrates for cAMP-dependent protein kinase which promotes steroid receptor functions*[150]. *There is a resemblance between function of the cAMP-dependent protein kinase and glucocorticoid receptor systems, both of which have kinase activity and play important roles in the mechanism of signal transduction.*

In a number of tissues, steroids have been shown to alter protein synthesis[151] *and cAMP regulation through the phosphorylation of the regulatory subunit of cAMP-dependent protein kinase*[106,152] *and affect the level of adenylate cyclase*[81,152] *and cAMP phosphodiesterase activities.*

A very important similarity between peptide growth factor receptors and steroid receptors is evident in respect to the transduction of the hormonal message to the genome. Although some difference in the structure and function exists between these two transduction systems, they are both functionally associated with hormone-stimulated kinase. In the case of peptide factor receptors, the protein tyrosine kinase is the cytoplasmic domain of the receptor[4,6,11]. *The signal from binding of the hormone to the extracellular domain is transmitted directly to the tyrosine kinase cytoplasmic domain which acivates the genes*[4,11]. *In the case of steroid hormones, covalent association of the receptor with the cell membrane is not required. Steroids are able to recognize their specific receptors within the respective target cells. This system, like the peptide growth-factor system, contains kinases that are involved in signal transductions. Does it seem that both steroid and protein growth factor signal-transduction systems consist of specific receptors and specific kinases associated with the receptors which phosphorylate both receptors themselves and other*

substrates? In both systems the purpose of receptor phosphorylation and other specific substrates is still unknown.

7.0. PROSPECTIVE STUDIES ON THE STRESS INDUCED CANCER. COOPERATIVE RESEARCH HEIDELBERG-BELGRADE

Starting in 1973, three prospective and intervention studies were initiated in Yugoslavia and Germany. A population-based sample of 16.250 men and 3.620 women serve as sampling frame for the assessment of patients who were known to suffer from neurotic symptoms, such as anxiety and chronic fear attacks, and apparently were under treatment (taking valium), but also those without specific therapies. Persons with the diagnosis schizophrenia, depression and with chronic hypertension (with and without treatment) were also included in the sample. Among the 16.250 males-interviewed in Heidelberg in 1973, 1.964 were selected for a more detailed interview. Most of the subjects were between 50 and 65 years old.

7.1. METHODOLOGY

The general method of selection was to choose the persons who suffered from different diseases in which catecholamine disturbances could create an important part of the pathology. As first sample, the number of persons with endogenous depression taking imipramine (for several years) was determined within the sampling frame. As soon as the study group of individuals with the specific diagnosis and treatment was composed, the matched comparison group with similar age (i.e., similar year of birth), identical diagnosis but without the specific treatment (i.e., without taking antidepressants) was identified. In this case, 321 pairs were obtained. By the same principle, one study group of persons with anxiety neurosis was selected, taking valium as sole treatment, whereas the comparison group was composed of persons with chronic anxiety, but without treatment. One group with hypertension and treatment by -methyl-dopa was compared with a matched control group without this therapy. A total number of 121 schizophrenics with phenothiazine therapy was compared with an identical, nonmedicated control group of schizophrenic persons. Another 11 such pairs were carefully selected for exposure to only one treatment scheme.

At the beginning of the study the patients were interviewed with a 70-item psychological questionnaire and medically examined in collaborating institutions with repeated measurements at regular intervals.

A fair number of psychological questions were asked, including those for long-

lasting hopelessness and depression due to adverse life events: a rational and antiemotional attitude: readiness for self-negation for the sake of harmonious relations with others and the fulfillment of one's duties: inability to relax and recover: readiness to expose oneself to unfavorable conditions; and long-lasting irritation and anger due to adverse life events. This evidence and experience have been systematized by the Grossarth typology distinguishing six behavioural types. A catalogue of questions on which the questionnaire was based refers to the typology published by Grossarth–Maticek et al.[2], *which with slight modification was used in this study. This typology*[10] *comprehends six types: Type 1 —Understimulation — inhibited expression of ego; Type 2 Overarousal - barriers in ego expression; Type 3 — Ambivalence — non-adequate expression of ego; Type 4 — Personal autonomy — expression of ego produces internal personal well-being: Type 5 — Rational antiemotional behaviour — using autogenic regulation to reach rational behaviour; and Type 6 — Inadequate — anticonforming expression of ego.*

The cancer-prone type showed an excess of inhibition (Type 1), the CHD-prone type an excess of excitation (Type 2), and Type 4 (the healthy type) showed an equilibrium. Type 3 showed a constant change from excitation to inhibition. This change to some extent protected it from excessive inhibition and excessive excitation, so that Type 3 probands were almost as healthy as Type 4 probands.

In 1986, 13 years after the beginning of the investigation a follow-up was carried out to obtain information about causes of death from death certificates, with emphasis on cancer, myocardial infraction and apoplexia cerebri.

7.2. PERSONALITY AND CANCER

The results of 3 major prospective studies, carried out one in Yugoslavia and two in Heidelberg, Germany, respectively are discussed. The Yugoslav population consisted essentially of the oldest inhabitant in every second house in a small village. The normal Heidelberg population consisted of a random sample of Heidelberg men and women within prescribed age limits, while the Heidelberg stressed group consisted of probands named as highly stressed members by the Heidelberg normal sample, and was essentially similar to that group in age, sex composition, and smoking habits. All groups consisted of healthy probands at the initiation of the study, and all were followed up for periods of 10 years.

Three major findings from these studies are important:

(1) the results obtained in this prospective study of the relation between the personality type and mortality caused by cancer and cardiovascular and cerebrova-

scular diseases provide evidence for the role of disorders whose pathogenesis creates the disturbances and imbalance of catecholamine in brain. In depressive untreated patients, who are characterized by a decrease of catecholamine in their brains, the higher mortality rate from cancer (24,6%) is obvious in comparison with other causes of death. Conversely, the long-term treatment of depressive patients with monoamine oxidase inhibitors (imipramine), which raises the level of catecholamine in brain, significantly reduced the mortality caused by cancer (5,2%).

Personality type predicts surprisingly accurately death and cause of death in all three studies. The differences between personality types are large, and highly significant statistically.

(2) Prediction of death and cause of death is approximately 6 times as accurate from personality type as from a history of smoking, cholesterol level, and blood pressure; all of these were ascertained at the same time as personality/stress.

(3) The effects of different risk factors for cancer are synergistic, not additive. Some 1.500 probands were selected from a much larger group, on the basis of 7 risk factors (smoking, stress, bronchitis, use of depressant drugs, exposure to car exhaust, genetics, and exposure to asbestos); these might be present singly, two at a time, three at a time... up to seven at a time. One group had none of these risk factors. There is a geometric progression in the incidence of lung cancer with the number of risk factors involved.

In this table, we are dealing with the results of a study in which we compare death rates of probands having 1, 2, 3, or all 4 of four different risk factors. The risk factors were 1) smoking (more than 20 cigarettes per diem, for over 10 years), 2) heredity (at least one first-degree relative suffering from, or died of lung cancer), 3) chronic bronchitis, and 4) stress, i.e., probands of Type 1 or 2. Not all combinations of risk factors could be found in sufficient numbers, but the data show very clearly the synergistic effects of multiplying risks.

It will be seen that in these probands, who were on average between 51 and 54 years old at the beginning of the study, 13 years later the combinations of 3 risk factors showed quite elevated death rates for lung cancer, varying from 7.6 through 9,8 to 20 percent. Combinations of four risk factors raised the death rate from lung cancer to 31% demonstrating the strong synergistic effect of multiplying risk factors.

7.3. EMPIRICAL RESULTS

1. Psychosocial stress in terms of high hopelessness, high antiemotionality etc.

increases the cancer incidence. In general it is 3-5 times higher in stressed patients (lung cancer 5 times). All probands of prospective studies were followed for period of 10 years.

2. Under stress the cancers of breast, cervix and corpus uteri represent more than 70% of all cancer incidence of women. In the population of men lung cancer is dominating 32%, rectum 12% and prostata 12% etc.

3. The prospective studies indicated that the incidence of cancer is related to the psychic constitution of the person and to personality, to the intrapersonal stress.

In the course of this study, the cancer-prone and coronary heart disease-prone personalities are defined :

a) the cancer-prone type (Type 1 or Type C) showed an excess of inhibition and has been described as appeasing, unassertive, overcooperative, overpatient, conflict avoiding and so on. These probands had very high incidence of cancer (40-50%) and high rate of death (30-40%).

b) Coronary heart disease-prone probands (Type 2 - Type A) showed an excess of excitation.

c) The healthy type (Type 4 - Type B) showed an equilibrium-personal autonomy. The expression of ego produces internal personal well-being.

4. The major findings from these prospective studies are following:

(1) Personality type predicts surprisingly accurately death and cause of death in all three prospective studies. The differences between personality types are large, and highly significant statistically.

(2) Prediction of death and cause of death is approximately 6 times as accurate from personality type as from a history of smoking, cholesterol level, and blood pressure; all of these were ascertained at the same time as personality/stress.

5. A group of 1500 probands selected from a much larger group, on the basis of 7 risk factors such as: smoking, bronchitis, depressants (drugs), exposure to car exhaust, genetics, asbestos and stress showed a geometric progression in the incidence of lung cancer with the number of risk factors involved.

6. The effects of different risk factors for cancer are synergistic with stress induction of cancer.

7. In depression untreated patients, who are characterized by a decrease of catecholamine in their brains, the rate of mortality from cancer was much higher by comparing with other causes of death.

8. Long-term treatment of depressive patients with monoamine oxidase inhi-

bitors (imipramine), which raise the level of catecholamine in brain, significantly reduced the mortality caused by cancer induced by stress.

9. In untreated patients with chronic fear attacks, essential hypertension, and schizophrenia in whom, more or less, increased and pronounced catecholaminergic stimulation of CNS persists relatively lower cancer incidence in the range of total mortality has been noticed (6,9%, 8,5% and 0,8% respectively).

10. It is conspicious that in schizophrenic patients a relatively low cancer mortality was found.

11. The lowest incidence of stress-induced cancer was found in persons of pcychological equilibrium - Type 4.

12. In the matched pairs analysis persons treated with valium for chronic fears generally exhibited a high rate of cancer deaths (20,4%), whereas untreated, age/matched men showed a rate of 6,9% cancer death within the 13 year's observation period.

13. Psychosocial stress is substantially associated with a low lymphocyte percentage (lymphopenia) which is a strong risk factor for cancer.

14. Cholesterol in circulation was reduced within a longer period before the outbreak of cancer was observed.

15. A decrease in immunity was observed in subjects exposed to chronic psychosocial stress.

16. In conclusion from the 13 year's follow up it is evident that psychological variables, in particular personality type, are important in mediating incidence and death from cancer induced by stress. Our results emphasize both importance of this typology and synergistic interaction between personality, viruses, chemical and pharmacological variables.

8.0. SPECULATIVE HYPOTHETIC MODEL ON MOLECULAR ASPECTS OF THE CARCINOGENESIS INDUCED BY LASTING PSYCHOSOCIAL STRESS

T h e m o d e l

The aim of the proposed hypothetical model is to integrate the events induced by emotional stress and to correlate them with the molecular mechanisms of carcinogenesis.

It is well established that steroids are involved in the regulation of gene expression: (a) by acting, via hormone-receptor complexes, on the genome of target cells and by regulating the rate of transcription of some particular genes and (b) by modulating, via specific receptor activation, the rate of phosphorylation[153,154] *and*

acetylation[155] *of chromatine proteins and the rate of demethylation of DNA sequences of particular genes*[156–158] *and (c) by acting at translation and posttranslation levels*[159]. *The corticoids regulate the rate and fidelity of translation by modulating the level of phosphorylation of ribosomal S6 and other proteins. The circulating corticoids regulate the activity of phosphoprotein kinases*[160]. *The increased activity of phosphoprotein kinases may enhance the activity of specific peptidases and proteases and may induce the activity of ornithine decarboxylase. These molecular events underlay the cancer promotion as it is shown in different living systems. There is a strong evidence that two critical steps in the formation of many human cancers, occuring in completely different tissues, may be artibuted to changes affecting what we might loosely call the* **ras**-*and* **myc**-*oncogenes function. The activation of celular* **ras** *protooncogenes (c-ras) can be achieved by mutation altering* **ras**-*oncogene product p 21, whereas the* **myc** *activation involves achromosomal translocation (t 8 : 14 or t 8 : 2). This could mean that one mutational event and one transposition or rearrangement event must occur in the natural genesis of many human cancers*[161].

The stress-induced levels of glucocorticoids may activate latent oncogenic polyoma virus[162]. *Cortisol is essential in inducing mammary tumor virus*[163–165] *in addition to corticosteroids the chemical carcinogens-epoxides may activate the viral oncogenes in host cells*[166]. *Both classes of agents may act in concert.*

Gonadal steroids levels are increased by emotional stress. Moderate increases in the circulating amounts of estrogen, progesteron and prolactin generally lead to an increased frequency of breast cancer[167]. *The prolonged estrogen treatment of the male syrian hamster results in the production of renal adrenocarcinomas*[168]. *Estrogen administration causes increased level of prolactin in the serum, and prolactin has been implicated in mammary tumorogenesis of rats and humans*[168]. *Serum cortisol level of women with the cancer of endometrium was slightly higher than that in normal women*[169]. *Therefore, theoretically, it is very likely that the stress-induced elevated levels of circulating corticoids may activate: (a) the dormant "Cancer" genes of normal cells; (b) the newly "initiated" transforming genes; and (c) the dormant "oncogenes" of inserted (integrated) viruses. Indeed, it is established in animals and humans that the elevated levels of cortisol or dexametason may activate "oncogenes" of transforming papiloma (DNA viruses) and those of mammary tumor virus (MMTV) in humans.*

If the action of steroid hormones is mediated by specific receptor, as it is that our model predicts a significant role of steroid receptor since the genes in target

cells are activated by hormone-receptor complex. The glucocorticoid receptor, as proposed by Kanazir (1980), is a heteromultimer — composed from distinct subunits. It is widely accepted that the steroid hormone-receptor complex recognizes specifically and binds to DNA sequences within or near promoter loci causing the activation of «enhancer-like elements» and, thereby effects the change in gene activity[170]*. The molecular events of these interactions are not clear yet. It is quite possible that the steroid hormone-receptor complex can, by interacting with «enhancers» within promoter sequences, regulate: 1. RNA-polimerase entry site (structure of chromatin) essential for gene transcription; 2. The conformational change of DNA caused by binding of complex to DNA, may act as a signal that can be transmitted over some distance. This may increase the number and stabilize the sites for RNA plymerase and initiation factor binding to DNA*[171].

In chromosomal rearrangements occuring during carcinogenesis a transposition of glucocorticoid-receptor binding sequences near c-oncogenes may occur. This may cause the activation of «dormant» c-oncogene by glucocorticoids and enhance the molecular events of carcinogenesis.

In an extensive study of steroid receptors in our laboratory, significant variations in the structure of steroid receptors in human malignant tissues were revealed. Thus, sucrose density gradient centrifugation revealed a wide range of sedimentation coefficients, from 2,5 to 8,5, for estradiol progesterone and glucocorticoid receptors prepared from human kidney cancers[172]*. The 4S component is the activated hormone-receptor complex translocated to cell nuclei, whereas 25 may be either 1B-complex (subunit of the receptor remaining in cytoplasm and acting as a regulator of cytoplasmic molecular events) or/and a degradation product. Our results suggest that in some human malignant tissues the steroid-receptors are structurally modified in such a manner that their regulatory functions may be enhanced.*

It is becoming evident that a subunit of the receptor (1B binder) is involved in regulation of phosphorylation of certain cytoplasmic proteins as well as in regulation of gene expression at translation and post-translation levels. This means that a subunit of the cytoplasmic hormone-receptor complex may regulate the activity of enzymes, such as proteases or phosphoprotein kinases, that underlie the promotion of carcinogenesis. Consequently hormone-receptor complexes may enhance the carcinogenesis at different levels of expression of genes. Thus the steroids, via increased and enhanced activation of specific receptors, due either to excessive production and secretion of steroids or to modified structure of the receptor, may mo-

dulate the expression of specific genes that may induce the neoplasmatic transformation of the cells.

The model we proposed also predicts that the risk of cancer incidence may be related to the synergistic action of chemical carcinogens, viral superinfection and emotional stress that can activate dormant either cellular or viral «oncogenes» and initiate a series of cascade phosphorylation which may enhance the appearance of cancers.

Stress-induced steroids and the activity of microsomal enzymes

In addition to activation of dormant «oncogenes» in a normal cell, our proposed model predicts that long lasting emotional stress may provoke the increased production of chemical carcinogens — epoxides from exogenous-dietary origin and/or endogenous surplus of circulating cholesterol and steroids that are produced in stressed organisms. The elevated amounts of steroids and cholesterol produced in stressed organisms act as inducers for the increased activity of microsomal mixed functions oxidases, that enhance the production of mutagenic and carcinogenic epoxides in liver, lung, kidney and other tissues.

Emotional stress and the immune competence of the organism

Both of the above mentioned pathways are operating at enhanced rates in cells of various tissues and may, in a synergetic way, act to decrease the immune competence of stressed organisms. The elevated levels of circulating corticoids and the increased amounts of carcinogenic epoxides may decrease the efficiency of immunologic surveillance of transformed cells. The function of T- and non-T- cells is inhibited by elevated levels of circulating corticoids allowing the dormant (latent) transformed cells to begin proliferating in stressed organisms, resulting in tumor appearance.

Consequently, the proposed model predicts that the interplay of all three pathways acting in a synergistic way, should result in carcinogenesis. Many of the postulates of our speculative model are only theoreticaly predicted but not experimentaly proved yet and require a comprehensive and systematic study.

8.1. PREDICTIONS

If the proposed molecular model is correct, some predictions may be made.
1. The progress of carcinogenesis induced by psychosocial factors may be influenced by psychotherapy. This seems to be the case. Data of Grossarth-Maticek et al. 1982,

indicate that about 80% of patients treated by psychotherapy showed a longer survival time than the control group. 2. The chronically used sedatives could according to the model prevent carcinogenesis or lower the incidence cancer. Data from our prospective studies[173] *indicate that derivatives of barbituric acid widely and chronically used as sedatives do lower the incidence of cancer. The mechanism(s) of this action is not well understood. Theoretically one could propose several alternative pathways for the action of barbiturates. Barbiturates may prevent the synthesis and release of corticotropin (ACTH) and gonadotropins. This may result in lowering the levels of circulating glucocorticoids and sex steroids. They induce an increase in microsomal epoxide hydrase activity which converts the intermediate arene epoxides formed by the monoxygenase system to the corresponding nonmutagenic transdihydrodiols*[174]*. Consequently barbiturates may lower the mutagenic epoxides formation as well as the level of circulating steroids. 3. The model predicts the enhanced activity of liver microsomal enzymes in stressed subjects. An increase in microsomal aryl hydrocarbon hydroxylase (AHH) activity was, indeed, demonstrated after cortisol administration*[175,176]*. 4. According to proposed model the elevation of circulating corticoids induced by chronic emotional (psychosocial) stress may alter the imunosurvailance by inhibiting both T- and B-lymphocyte and macrophage-mediated functions. In our prospective studies, the marked lymphopenia was observed. 5. One of the most promissing predictions of the model is that the prevention of enhanced synthesis and secretion of steroids and over-expression of cellular and integrated viral oncogens (***myc*, *ras*-oncogenes**) would decrease the incidence of cancer. Recently it was reported that dihidroxymetabolite of vitamin* D_3*(1,25(OH)*$_2$ D_3*) increases the survival time of mice injected with myeloid leukemia cells. In this case* **c-myc** *transcription is reduced to 50% of initial values within 4 hours*[177]*. This derivative of vit* D_3 *appears to have significant clinical potential in the treatment of leukemic patients. In addition, antiproliferative effects of retinoic acid and vit A have been demonstrated in breast cancer cell lines*[178]*, leukemia cells*[179]*, embrional carcinoma and melanoma cells*[180]*.*

9. DISCUSSION

Bearing in mind that cancer is a disease of multifactorial etiology and that carcinogenesis is a multi-step process, the explanation of the high correlation of cancer and given psychosocial as well as neurological determinants includes at least three levels: brain homeostasis, neuroendocrine transduction, and target cell DNA

response. (Mutation and regulation of genes expression, especially genes involved in signal transduction).

Many years ago it was pointed out that brain homeostasis is maintained by different systems regulating behavior through integration of basic neural processes of excitation and inhibition. Excitation and inhibition as basic functional elements of the nervous system represent two sides of a unique process and determine by their balance the character and direction of the regulatory adaptive mechanisms of human and animal organisms. Both processes participate in various combinations dependent on level and form of the regulation of vitality important functions of the organism.

Certain experimental and clinical data indicate that stress is involved in the genesis of behavioral and psychosomatic impairment, via its disruptive effects on brain monoamine neurotransmitters[12]*. More evidence implicating DA deficits in the etiology of depression originated in «learned helplessness» and «behavioural dispair» models of depression.*

The altered brain homeostasis caused by emotional stress as well as in other pathological conditions, followed by decreased level of catecholamines, particularly in the hypothalamus[13]*, produces remarkable changes in neuroendocrine transduction increased stimulation of ACTH and gonadotropins, followed by steroidogenesis and elevation of both corticoids and sex steroids.*

Short term exposure of mature male rats to the above stressors led to a decrease in type II GR mRNA levels in the hippocampus, while it did not affect type II GR mRNA levels in the cerebellum. On the other hand, repeated (for 2 weeks) daily exposure of the animals to the same stressors resulted in a remarkable increase in type II GR mRNA levels in both the hippocampus and the cerebellum. Thus, in the acute phase of the organism's response to stress, glucocorticoid receptors are specifically down regulated in the hippocampus. In contrast, during adaptation they are upregulated not only in brain areas directly involved in the brain-pituitary-adrenal axis, such as the hippocampus, but in other brain regions as well, such as the cerebellum. (E. Kitraki, N. Tritos, and F. Stylianopoulou, Stress has an effect on the expression of the type II glucocorticoid receptor gene in the rat brain).

Finally, all those functional and structural changes mediated by steroids alter the regulation of gene expression and the target cell DNA with multiple and multi-step changes over a broad spectrum — from the initiations of mutations, insertion of viral oncogenes, viral promotors, activation of C-oncogenes, to the activation of dormant cancer cell, tumorogenesis, progression of cancer, including the subse-

quent increases of cancer incidence and mortality, as was described in detail previously.

The described integrity of the system, brain homeostasis — neuroendocrine transduction — target cell DNA, plays an active part in the general adaptation and survival of the organism. The pathological conditions associated with excessive influences of stress factors on the organism, other pathological conditions with a decreased level of endogenous amines in the brain and increased level of corticoids in the body, produce an increased activity of the adaptive system due to deterioration of the mechanism of autoregulation. The system becomes more vulnerable under the influence of stress. The continuous regulatory deficiency in adaptive homeostasis triggers unbearable functional and metabolic — including immunological — disturbances, important for promotion of carcinogenesis, progression of tumor growth and metastasis. Consequently, an increased level of catecholamines (in schizophrenia, essential hypertension) operates in a different direction in adaptive homeostasis, triggering the system mechanisms responsible for the suppression of carcinogenesis.

There is a common opinion among tumor immunologists that antitumor reactivity of immune lymphocytes is dependent principally upon cytotoxid T cells that recognize tumor-associated antigens in conjunction with histocompatibility complex antigens in cell membranes of antigen-presenting cells (macrophages dendritic cells).

Also, a large body of evidence exists to show that tumors are more aggressive in immune-deficient hosts, and that various forms of immune system stimulation can modify or control cancer progression[5]*. As our understanding of the immune system as an interactive network of dialogues within subsets of immune-competent cells and tumor cells becomes more sophisticated, multiple mechanisms of immune action against tumors have been hypothesized.*

The neuroendocrine axis is being recognized increasingly as an important component of the immune response. The neuroendocrine apparatus is involved in short — and long-range communication by means of paracrine, transmitter-like, modulatory and neurohormonal types of messages. Soluble mediators are amines, peptides like enkephalins, bradykinin, substance P, bombesin or short-chain lipids like prostaglandins, thromboxane and leukotrienes. There are receptors for these substances on lymphocytes and neurons and they employ both the cyclic AMP and PI transmembrane signalling mechanisms.

Although early changes of a transformed cell clone may not be influenced by

immunological processes, the growth of tumors and their metastatic spread might well be affected by the body's defense system. It becomes common sense among immunologists that the immune system is not only modulated by antigens, but can also be activated by neuro- and immunopeptides as messengers in a neuroimmune axis. The relationship of external events (e. g., bereavement), overt behaviour in response and coping attempts, and the internal immunological and neuroendocrine state of the individual might become descriptive within the new interdisciplinary field called cognitive science.

10.0. CONCLUSION

Malignant tumors arise from a protracted sequence of events which is not yet understood. It is evident that cancer is caused by many agents all of which act in a common way, ultimately damaging the structure and function of cellular genome (DNA).

On the basis of a considerable variety of evidence on gene mutation, deletion amplification, and transpositions are implicated in the activation of proviral or cellular oncogenes and the genesis of human and animal tumors. These lesions may be present even in oncogenes carried by retroviruses. The function of oncogenes is not yet well understood. The products of immortalizing oncogenes (myc, fos) are found almost exclusively in the nucleus and are thought to act by inducing transcription of other silent genes. The products of transforming oncogenes (ras, src, and others) are associated with the cell surface and signal transducing systems. These genes may be «turned on» by immortalizing oncogene products or other regulatory factors such as steroid hormones. As has already been mentioned, the steroid hormones regulate gene expression and thereby the processes of cell metabolism, differentiation, and growth . It is also well established that neuroendocrine hormonal disorders leading to increased levels of circulating steroids, as well as the ectopic oversecretion of steroid hormones, may generally increase cancer incidence or maintain malignancy in humans. The pathway of the action of oversecreted steroids may be neuroendocrine, paracrine, and autocrine. Thus, the excess of steroid hormones may, through neuroendocrine and paracrine pathways, cause cancer in distant target cells, whereas autocrine secretion may maintain the malignant state. The excess of steroid hormones may cause cancer in several ways. It may activate latent proviral oncogenes or silent cellular protooncogenes; stimulate microsomal enzymes that convert chemical procarcinogens into active carcinogens (these may originate even from overproduced endogenous cholesterol and/or circulating steroids); and

depress immunologic surveillance of latent transformed cells. All actions of steroid hormones are mediated by specific steroid-receptor complexes. It is widely accepted that steroid receptors, involved in the stimulation of gene expressions, play important roles in malignant transformation, but steroids can also inhibit gene expression. While they play an important role in malignant transformation, they can also prevent cancer, stimulate the reversion of the malignant phenotype, and be used for the therapy of steroid hormone-dependent cancers.

It is well established that the action of steroid hormone-receptor complexes on unaltered target genes depends on the structure and function of the specific receptor. Any modification of the steroid receptor, due to frameshift or nonsense mutations and/or deletions within the steroid receptor structural gene, can markedly alter the structural properties of the receptor. These changes may result in truncated receptors whose activity is no longer affected by steroid hormones. The truncated steroid-receptors may act as constitutive transcription factors that recognize the hormone responsive elements of target genes. Structurally and functionally altered steroid-receptors have been found in a wide variety of malignant tissues. Lower molecular-weight receptor forms have been detected in cancerous tissues. The structural changes may reduce the half-life of the receptor, its solubilization, or produce some partial, controlled proteolysis.

*It should also be stressed that a high level of homology between steroid-receptor genes and some oncogenes has been demonstrated. Thus, there is a significant homology between c- erb*A *and v- erb*A *oncogenes, and steroid receptors. It was noticed that the erb*A *gene product may bind some steroid hormones. It is very likely that the truncated steroid receptor may behave as the product of an oncogene and as such may have a role in carcinogenesis similar to that of the erb*A *gene product.*

Modifications of the steroid-receptor structure may affect other receptor functions such as activation, translocation to the nucleus, interaction with the hormone responsive sites of the promoters of hormone inducible genes, as well as its role in signal transduction. Steroid receptors are tightly associated with a protein kinase or have intrinsic kinase activity. Any change in this kinase activity may alter the normal receptor in hormone binding and signal transduction and may thereby activate some silent oncogenes. All of the accumulated evidence makes it certain that we need more information about the structure and function of steroid receptors and their role in carcinogenesis. Knowledge of such information may have important practical value for diagnosis and prognosis as well as choosing effective hormonal and antihormonal therapy for cancers.

Our results may be, briefly, sumarized as follows: 1. psychosocial stress in terms of high hopelessness, high antiemotionality etc. has a strong relevance for cancer incidence; 2. psychosocial stress significantly enhances the efficacy of the most important physiological risk factors for cancer; 3. few of the theoretically possible molecular mechanisms and a highly hypothetical model, by which chronic emotional stress could increase the cancer incidence, as well as molecular aspects of prevention, at least as cellular level, are in this review briefly elaborated. As it appears the elevated circulating concentrations of corticoids and gonadal steroids as well as structural and functional alterations of their specific receptors seem to underlie the molecular mechanisms related to the activation of oncogenes either by mutation or/and by over expression. Attempts are made to test the proposed model in animal experimental conditions.

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