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Cell cycle checkpoints: the role and evaluation for early diagnosis of senescence, cardiovascular, cancer, and neurodegenerative diseases

Review Article

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Summary. Maintenance of genomic integrity is critical for prevention of a wide variety of adverse cellular effects including apoptosis, cellular senescence, and malignant cell transformation. Under stress conditions and even during an unperturbed cell cycle, checkpoint proteins play the key role in genome maintenance by and mediating cellular response to DNA damage, and represent an essential part of the ''cellular stress response proteome''. Intact checkpoint signal transduction cascades check the presence of genome damage, trigger cell cycle arrest, and forward the information to the protein core of cell cycle machinery, replication apparatus, repair, and/or apoptotic protein cores. Genetic checkpoint defects lead to syndromes that demonstrate chromosomal instability, increased sensitivity to genotoxic stress, tissue degeneration, developmental retardation, premature aging, and cancer predisposition that is most extensively studied for the ATM-checkpoint mutated in *Ataxia telangiectasia*. Tissue specific epigenetic control over the function of cell cycle checkpoints can be, further, misregulated by aberrant DNA methylation status. The consequent checkpoint dysregulation may result in tissue specific degenerative processes such as degeneration and calcification of heart aortic valves, diabetic cardiomyopathy, hyperhomocysteinemic cerebrovascular, peripheral vascular and coronary heart diseases, neurodegenerative disorders (Alzheimer and Parkinson diseases, amyotrophic lateral sclerosis, glaucoma), and accelerated aging frequently accompanied with cancer. This review focuses on the checkpoints shown to be crucial for unperturbed cell cycle regulation, dysregulation of which might be considered as a potential molecular marker for early diagnosis of and therapy efficiency in neurodegenerative, cardiovascular and cancer diseases. An application of the most potent detection technologies such as ''Disease Proteomics and Transcriptomics'' also considered here, allows a most specific selection of diagnostic markers.

Keywords: Stress response proteome – Cell cycle checkpoints – Epigenetic control – Cardiovascular and cancer diseases – Senescence and neurodegeneration – Early diagnosis and follow-up – Disease proteomics and transcriptomics

1. Stress response and the role of checkpoints

Stress response is the clue ability of each organism which decides over adaptation quality and survival under extreme as well as routine conditions. At the organismal level, stress response is controlled by hormones (Charmandari et al., 2005). Cellular stress response is frequently non-specific to the origin of stress factors causing the response; it is controlled by the quality/deformation of the essential cellular macromolecules as reviewed by Kultz (2005). The capacity of the cellular stress response depends on the pool of proteins expressed at the time point of stress application and on additionally activated ones in response to the stress. This pool of proteins is called ''Stress Proteome''. It has been shown that proteins involved in key aspects of the cellular stress response are highly conserved in all organisms tested such as human beings, yeasts, eu- and archae-bacteria, and the ''Minimal Stress Proteome'' of cellular organisms consists mainly of the following functional groups (Kultz, 2003):

- ''Energy metabolism and ATPases/ABC-transporters'',
- ''Translation regulation, protein synthesis and degradation'',
- ''Redox regulation'',
- ''Nucleic acid metabolism'',
- ''DNA damage sensing/repair and cell cycle control'',
- ''Basic transcription, replication, and RNA processing'',
- ''CoA metabolism'', and
- ''Molecular chaperones''.

DNA damage and protein oxidation represent the most severe deformations of cellular macromolecules, and trigger plenty of intra- and extra-cellular signaling cascades. Thereby, genotoxicity is the most fatal consequence of any kind of stress factor, such as the most ubiquitous emotion stress, smoking and alcohol consumption (Korystov, 1997; Irie et al., 2001), hyperhomocysteinemia (Sachdev, 2005), folate and taurine deficiency (Tomei et al., 1990; Chern et al., 2001; Golubnitschaja et al., 2003), UV- and ionizing irradiation (Brown et al., 2003), fluctuating blood glucose concentration (starvation, *Diabetes mellitus*, etc. (Massague, 2004; Golubnitschaja et al., 2006a), asphyxia/ischemia/stroke/reperfusion (Dell'Anna et al., 1997; Didenko et al., 2002; Cherubini et al., 2005), genetic syndromes such as Down syndrome (Midorikawa and Kawanishi, 2001) neurodegenerative diseases such as Huntington, Alzheimer, and Parkinson diseases, glaucoma (Chuang, 2005; Migliore et al., 2005; Hegde et al., 2006; Moenkemann et al., 2005), etc.

Under stress conditions and even during an unperturbed cell cycle, checkpoint proteins play the key role in genome maintenance by and mediating cellular response to DNA damage, as it is schematically shown in Fig. 1. Checkpoint signal transduction cascades check the presence of genome damage, trigger cell cycle arrest, and forward the information to the protein core of cell cycle machinery, replication apparatus, and either repair or apoptotic protein cores (Massague, 2004). Damaged DNA in humans is

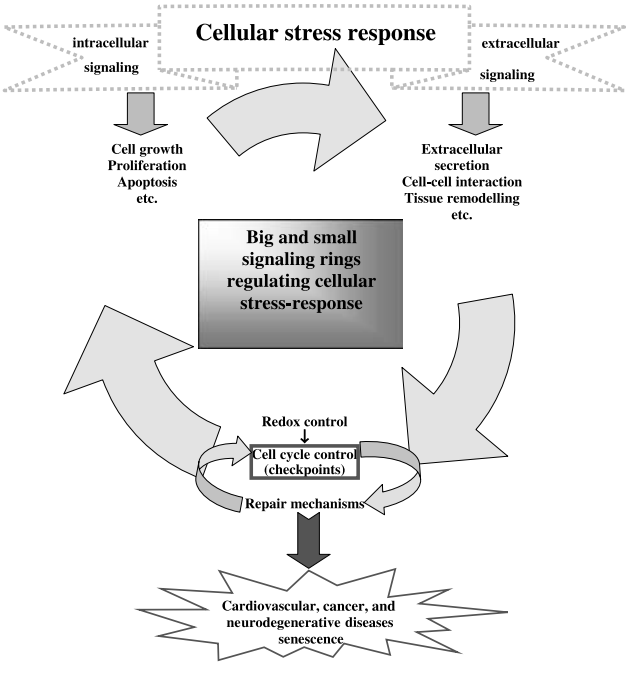

Fig. 1. The role of cell cycle checkpoints in cellular stress response

detected by a cascade of sensor proteins (HUS1, RAD1, RAD9, RAD17, RAD26), which transmit signals via ATR to CHK1, or by the other cascade of sensor proteins (NBS1, BRCA1, RAD50, etc.), which transmit signals via the ataxia-telangiectasia mutated (ATM) protein kinase to CHK2 (Kawabe, 2004). Double-stranded DNA (dsDNA)-breaks activate ATM which, in turn, modulates numerous signalling pathways via target phosphorylation that either enhances or represses activity of ATM-dependent proteins. ATM-mediated activation of cell-cycle checkpoints in response to (dsDNA)-breaks include following targets: P53, CDC25A, CDK2, CHK2, $P21^{WAF1/CIP1}$ (CDKN1A) in G_1/S phase transfer, and CHK1, CHK2, 14-3-3 proteins in G_2/M phase transfer of cell cycle (Xie et al., 1998; Shiloh, 2003; Kawabe, 2004; Pereg et al., 2006). Checkpoints response to single-stranded DNA (ssDNA)-lesions and DNA replication blockage is triggered by ATR-dependent pathways (Bakkenist and Kastan, 2004; McGowan and Russel, 2004; Levitt et al., 2005). By this way, checkpoint signal cascades response to a variety of stress conditions by coordinating of cell cycle arrest/promotion, various pro-apoptotic factors, the DNA-repair machinery, and oxidative-stress response genes (Massague, 2004). Genetic defects affecting DNA damage response pathways lead to syndromes that demonstrate chromosomal instability, increased sensitivity to genotoxic stress, tissue degeneration, developmental retardation, premature aging, and cancer predisposition. The most prominent genomic instability syndromes are *Ataxia telangiectasia* and *Ataxia telangiectasia*-like disease, Bloom's, Cockayne's, Nijmegen breakage, Rothmund-Thompson, Werner's syndromes, Fanconi's anemia, Trichothiodystrophy, and *Xeroderma pigmentosum* as reviewed by Shiloh (2003).

Recent works clearly showed that dysfunction of checkpoints uncoupled from cellular apoptotic/repair mechanisms leads to severe consequences such as human genetic syndromes, cardiovascular and cancer diseases, and senescence. Can we use cell cycle checkpoints as molecular markers for early diagnosis and prognosis of disorder severity?

2. Potential application of checkpoints genes as molecular markers for early diagnosis of cardiovascular diseases

2.1 Checkpoints in heart diseases

Aortic valve degeneration is one of the leading causes of heart diseases in adults. The mechanisms underlying aortic valve degeneration are largely unknown. Cardiac tissue

responds to a variety of stimuli by hypertrophic growth. Molecular mechanisms resulting in the hypertrophic response indicate similarity and overlap with those involved in both cell growth and death. This hypertrophic growth has been shown to be associated with the re-activation of the fetal gene program in cardiac cells (Liu and Olson, 2002; Bar et al., 2003), the clue of which is the positive regulation of the cell cycle progression. This switch in program might be crucial for the myocardial cell regulation. Such growth stimulation is responsible for the up-regulated activity of cyclin-dependent kinases (CDKs) that consist of a kinase core and an associated cyclin subunit acting as the positive regulator (Sherr, 1994). Thereby different CDK inhibitors keep the negative control over CDKs activities. CDK inhibitors are classified on the basis of their sequence homology and substrate specificity. Recently a novel cardiac helicase CHAMP that inhibits cell proliferation and cardiac hypertrophy was described (Liu and Olson, 2002). The CHAMP-dependent inhibition of cardiac hypertrophy is accompanied by an obligatory up-regulation of the cyclin-dependent protein-kinase inhibitor $P21^{WAF1/CIP1}$, a 21-kDa protein, which is a member of the CIP/KIP family (Sherr, 1994). Furthermore, a targeted over-expression of $P21^{WAF1/CIP1}$ prevents cell enlargement and suppresses a specific gene expression of the cardiac hypertrophy markers in the cell population in vitro (Tamamori et al., 1998) indicating the key role of $p21^{WAF1/CIP1}$ in the regulation of the hypertrophic response. Recently clear correlations among

- the well coordinated regulation of $p21^{WAF1/CIP1}$ and 14-3-3 sigma – checkpoint genes arresting cell cycle in G_1 and G_2 phases, respectively,
- modulation of the density of valvular cell population, and
- progressive calcification of native aortic valve tissue

were reported using "Disease transcriptomics" technology (Golubnitschaja et al., 2006b).

Cell cycle control obviously plays a key role in the maintenance of cardiac cell population: high rates of cell death have been shown to be physiological in normal adult human heart and those of mice and rats (Nadal-Ginard, 2001). The physiological expression of $p21^{WAF1/CIP1}$ shows a gradual increase during development in both rat and man, becoming maximal in adulthood (Burton et al., 1999). A direct link between the Bcl-2 dependent down-regulation of $p21^{WAF1/CIP1}$ and an increased myocyte density in the left ventricle has been shown recently in experimental work with transgenic mice (Limana et al., 2002). These findings are in agreement with the data demonstrating that down-regulation of both checkpoint genes $p21^{WAF1/CIP1}$ and 14-3-3 sigma correlates well with increasing cardiac cell density and calcification appearance in aortic valve tissue (Golubnitschaja et al., 2006b). Interestingly, the number of macrophages does not correlate with the cell density, whereas both the increased cell density and well coordinated down-regulation of $p21^{WAF1/CIP1}$ and 14-3-3 sigma gene expression were found to be characteristic for calcification. Therefore, the coordinated double-check of DNA quality and cell proliferation in valvular cells might be efficient only in non-calcified tissue, whereas in the calcified one this control becomes coordinately suppressed in both G_1 and G_2 dependent checkpoints. These findings give further evidence that the efficiency of cell cycle control in human non-calcified valvular tissue depends not only on the positive/negative CDKs regulation in the G_1 phase but also on the coordinated regulation of both G_1 and G_2 dependent checkpoints, that has not been considered from the view point of molecular pathomechanisms of valve calcification until now. Recent in vitro experiments on rat cardiac fibroblasts showed, that a target up-regulation of inhibitors for G_1 dependent CDKs effectively suppresses the DNA synthesis and may decrease a potential risk of cardiovascular diseases (Mercier et al., 2002).

The dissociation of $P21^{WAF1/CIP1}$ from the CDK complexes correlates well with the activation of CDK2, CDK4, CDK6, and the release from cell cycle arrest, whereby the number of cardiac cells in S phase rises considerably (von Harsdorf et al., 1999). Further, in contrast to P16 (a specific inhibitor of CDK4/6), the "universal" CDKs inhibitor $P21^{WAF1/CIP1}$ was shown to be able to block an E2F-1-induced G_1 exit completely (Akli et al., 1999). However, E1A binding activity to target protein complexes has effects on the cell cycle progression beyond those produced by E2F-1 alone, and can drive S-phase entry that is resistant to $P21^{WAF1/CIP1}$ (von Harsdorf et al., 1999). These facts explain the necessity of the coordinated regulation of both G_1 and G_2 dependent checkpoints, in order to keep the control over the cell population maintenance in cardiac tissue. Blockade of cell cycle progression results in a prolonged resistance to macrophage invasion and foam cell deposition (Mann et al., 1997). Therefore, it is likely that reduced cell cycle control in valvular tissue leads to the increased macrophage invasion that, in turn, can contribute to non-physiological calcification by both triggered unspecific inflammation and NO-toxicity (Schmidt and Walter, 1994; Zhuang and Wogan, 1997; Lutgens et al., 1999; Sanders et al., 2001; Frangogiannis et al., 2002). The coordinated activation of

both G_1 and G_2 dependent checkpoint genes may be, therefore, an attribute of the valvular tissue resistance against the calcification processes. Molecular events leading to valve calcification are schematically shown in Fig. 2, and should be taken into consideration, when designing therapeutic prevention of pro-calcification molecular pathways in human heart.

2.2 Altered expression of checkpoints in diabetic cardiomyopathy

Many diabetic patients suffer from cardiomyopathy, resulting in heart failure and increased mortality, even in the absence of vascular disease (Liang et al., 2002). More than 50% of patients with type 2 diabetes have coronary heart disease, related to silent ischemia and autonomic denervation of the heart. Little is known about molecular mechanisms leading to the degeneration of cardiac tissue under diabetic conditions. Hyperglycemia-induced oxidative stress and subsequent DNA damage is speculated to contribute to the pathogenesis of diabetic complications in both diabetes types 1 and 2 (Dandona et al., 1996; Hinokio et al., 1999; Sampson et al., 2001; Sardas et al., 2001). A repair capacity towards increased production and damage effects of reactive oxygen species (ROS) in diabetic patients or corresponding animal models is now under intensive investigation as a parameter or even biomarker of differential cell resistance towards diabetic complications (Hinokio et al., 1999; Sampson et al., 2001; Taniguchi et al., 1996; Leinonen et al., 1997; Krauss et al., 2003). Noteworthy, in the early phase of diabetes the heart is more resistant to ischemic damage than the non-diabetic heart (Tosaki et al., 1996; Paulson, 1997). Therefore, it has been hypothesized that oxidative stress conditioned by hyperglycemia may be associated with an increased density of damaged cardiomyocytes followed by P53-dependent induction of cell cycle arrest in cardiomyocytes and consequent reduction in cell population with a concomitant remodeling of heart tissue (Golubnitschaja et al., 2006a). Indeed, in the early phase of STZ-induced insulin-dependent diabetes both cell cycle checkpoints $p21^{WAF1/CIP1}$ and 14-3-3 sigma become activated in cardiac tissue compared to the non-diabetic heart. Furthermore, a repair of damaged DNA is strongly promoted in diabetic cardiomyocytes, since the measured levels of repaired DNA molecules have been shown to be even higher than those of non-diabetic cardiomyocytes. These findings suggest that arrest of cardiomyocyte proliferation under diabetic conditions might be triggered in both G_1 and G_2 phases of the cell cycle, and accompanied by extensive DNA repair provided by a complex, both G_1- and G_2-dependent repair machinery in the early phase of the disease. This conclusion is of high importance, since it could explain the observed but not yet understood increased resistance of the heart towards ischemia/reperfusion in early diabetes compared to the non-diabetic heart. Further observations of disappearing of this beneficial effect with the duration of diabetes proposes a dual role of cell cycle checkpoints under diabetic conditions: in early diabetes an increased expression of both $p21^{WAF1/CIP1}$ and 14-3-3 sigma genes has a beneficial effect activating DNA repair processes triggered by cell cycle arrest, and, therefore, preventing replication of damaged DNA. On the other hand, the double cell cycle arrest ultimately inhibits replication of cells which consequently accumulate in the G_1 and G_2 phases; this potentially might lead to the myocardial tissue degeneration well-documented in later phases of diabetes. The proposed molecular events leading to diabetic cardiomyopathy fit also well to the general scheme of cardiac tissue degeneration given in Fig. 2. Indeed, in contrast to early stages, the advanced stages of the diabetic cardiac hypertrophy and fibrosis are accompanied with an inactivation of 14-3-3 protein (Gurusamy et al., 2005).

Further investigations of checkpoints expression in cardiomyocytes of diabetic rats demonstrated that the basic level of regulation – namely gene methylation status – is involved (Moenkemann et al., 2002). There is a strong epigenetic control over gene expression of both $p21^{WAF1/CIP1}$

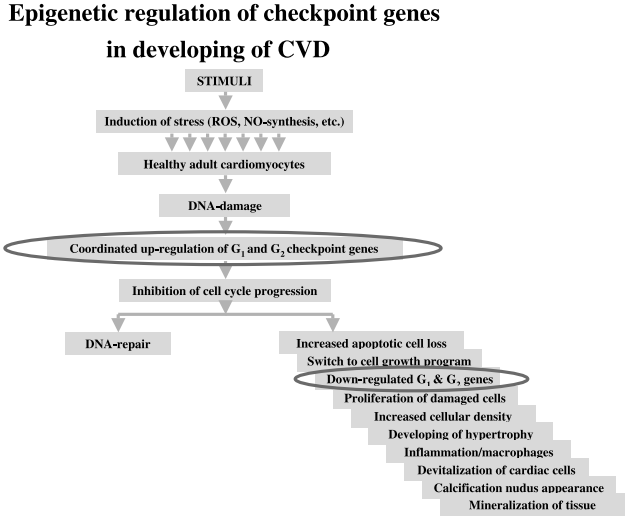

Fig. 2. Schematic presentation of the cascade of molecular events leading to valve degeneration and mineralization of cardiac tissue. Coordinated up-regulation of G_1 and G_2 checkpoint genes was demonstrated in degenerated but non-calcified valves. In contrast, the expression of cell cycle checkpoints was completely suppressed in calcified tissue (Golubnitschaja et al., 2006b)

and 14-3-3 sigma: methylation status of abundant CpG islands located in a promoter region is known to have an important regulatory effect on their expression (Allan et al., 2000; Ferguson et al., 2000). The mechanisms of gene silencing by methylated cytosin are varied among promotors. The most generally reported mechanism is the repression of transcription by methyl-CpG-binding proteins (MeCP1 and MeCP2) that bind DNA in a sequence independent manner. Thereby, an activation of methyl-CpG-binding proteins results in alternating the chromatin structure and preventing the transcriptional factors like Sp1 from DNA binding (Lewis et al., 1992; Tate et al., 1996; Simmen et al., 1999).

Some therapeutic approaches to prevent both diabetic and non-diabetic cardiomyopathy are currently under consideration. Increasing evidence suggests that dietary taurine supplementation reverses cardiomyopathy in animals underwent taurine depletion (TD) and also confers beneficial effects in patients with heart failure (Schaffer et al., 2000a; Sole and Jeejeebhoy, 2002; Pion et al., 1987; Azuma et al., 1992). Several mechanisms have already been proposed to account for the beneficial effect of taurine in heart failure: improvement of tissue osmoregulatory properties and ion transport mechansims (Labudova et al., 1999a; Schaffer et al., 2000a; Suleiman, 1994; Moenkemann et al., 1999) promotion of body fluid homeostasis by pronounced natriuresis and diuresis effects (Mozaffari and Schaffer, 2001), positive ionotropic effect by regulating $[Ca^{2+}]_i$, $[Na^+]_i$ and Na^+/Ca^{2+} exchanger activity (Azuma et al., 1992; Schaffer et al., 2000a, b), and attenuation of angiotensin II mediated events such as induction of cardiac hypertrophy, myocardial remodeling and volume overload (Schaffer et al., 2000b). Oxidative stress is proposed to be the pathogenic mechanism linking TD to the ultimate manifestation of cardiomyopathy. Similarly to diabetic rats, the taurine depletion causes DNA damage accompanied by up-regulation of P53, $P21^{WAF1/CIP1}$ and 14-3-3 sigma expression in cardiomyocytes (Golubnitschaja et al., 2003). Since TD is associated with the development of cardiomyopathy, the relevance of these molecular events to the ultimate manifestation of cardiomyopathy caused by both *Diabetes mellitus* and TD represents a fertile area for further investigation.

2.3 Hyperhomocysteinemia as an important independent risk factor for cardiovascular diseases and potential moderator of tumor activity

Hyperhomocysteinemia (Hhcy) is associated with the development of cerebrovascular, peripheral vascular and coronary heart diseases (Wilcken et al., 1983; Brattstrom et al., 1984; Boers et al., 1985; Malinow et al., 1989, 1996; Coull et al., 1990; Clarke et al., 1991; Graham et al., 1997). Elevated plasma levels of homocysteine (Hcys) can result from genetic (such as mutations in 5,10-Methylenetetrahydrofolate reductase – the key enzyme in both folate and homocysteine metabolic pathways) or nutrient-related disturbances (e.g. folate deficiency) in the transsulphuration or remethylation pathways for Hcys metabolism (Frosst et al., 1995; Akoglu et al., 2004). Hcys significantly stimulates DNA synthesis, promotes vascular smooth muscle cells (VSMCs) growth inhibiting endothelial cell growth and stimulating proliferation of arterial tissue, and consequently promotes the vessel wall thickening potentiating calcification of aortic smooth muscle cells (Lubec and Szekeres, 1994; Tsai et al., 1994; Li et al., 2003). What are the molecular mechanisms of this selective promotion of VSMCs proliferation? Lubec et al. demonstrated the ability of Hcys to activate the cell cycle progression by stimulation of aortic cyclin-dependent kinase at the transcriptional level in a dose-dependent manner (Lubec et al., 1996). This important finding has been later confirmed by numerous further experimental data demonstrating Hcys mitogenic effect on VSMCs with strong proliferative response on the luminal side of the vessel wall tapering, however, towards the adventitia, and showing also a significant inhibition of DNA synthesis and of proliferative effect by nutritional folate supplementation (Carmody et al., 1999; Chen et al., 2000; Buemi et al., 2001; Kartal et al., 2005). Further studies demonstrated induced B and T lymphocyte proliferation by increased Hcys concentration levels; the underlying cell cycle progression mechanisms involve PKC, p38 MAPK and NF-kappaB activity (Zhang et al., 2001, 2002). This might be the further mechanism potentiating chronic inflammatory progression of atherosclerosis with Hhcy.

Recently achieved results represent a functional link of Hhcy associated cardiovascular and cancer diseases: 5,10-Methylenetetrahydrofolate being the key metabolite in both folate and Hcys metabolic pathways, is the main modulator of growth-promoting effects of Hcys as well as anti-proliferative ones of folate in colon cancer cells (Akoglu et al., 2004). Hhcy-induced alterations of extracellular-matrix (ECM) metabolism can, further, contribute to the progression of both cardiovascular and cancer diseases: Hhcy increases the secretion of elastolytic metalloproteinases-2 and -9 and tissue inhibitor of metalloproteinases-1 (Yang and Zou, 2003; Chaussalet et al., 2004). These alterations can potentially trigger a

non-physiologic deposition of ECM-elements and advance a tumor metastatic activity. Hhcy research provides us with a further important functional link between cardiovascular and cancer diseases: Hhcy is associated with altered overall DNA methylation status in different cell types (Hultberg et al., 2000; Castro et al., 2003) representing the basic mechanism of the epigenetic control, particularly, over cell cycle regulation. Further studies are required before the true role of Hcys and Hhcy in the regulation of overall DNA methylation status can be clearly evaluated. This can dramatically further our knowledge about the origin of cardiovascular and cancer diseases.

3. Checkpoints in cancer: differential gene expression, epigenetic control and cell cycle progression, potential early diagnosis and therapy monitoring

Mammalian cell cycle checkpoints activation has been proposed as the key tumor-suppressor mechanism that should prevent the mutation accumulation that usually drives oncogenesis (Hartwell and Kastan, 1994). Mutations in and/or complete loss of even one checkpoint results in the pronounced predisposition to cancer diseases, as it has been well documented in the case of *Ataxia telangiectasia*, the human genomic instability syndrome characterized by a mutated non-essential cell cycle modulator – the ATM protein kinase as reviewed by Shiloh (2003). This checkpoints-associated increased tumour predisposition might be developed either during embryogenesis or can be, further, caused by some pathologic conditions in child- or adulthood such as highly increased stress of any kind and DNA modifying conditions such as altered DNA methylation status, DNA damage, viral infections etc. Thereby, apart from increased tumor predisposition, inborn checkpoint mutations are also associated with diverse and usually well-detectable abnormalities like growth and mental retardation, facial dysmorphism, immune dysfunction, etc., commonly found in other syndromes associated with an impaired response to DNA damage. These abnormalities are well-documented in the human genetic disorders ''Seckel syndrome'' with inborn defect of *atm*-related *atr*-checkpoint gene, and indicate a broad application of checkpoints in organismal development and functions (Alderton et al., 2004).

It is more complicated to predict a non-inborn tumor predisposition arose in child- and adulthood due to any DNA modifying events. Similarly to inborn checkpoint mutations, the potential tumorigenesis selects for genetic and epigenetic changes, allowing evasion of anti-proliferative and apoptotic mechanisms, when the arising tumor acquires genetic instability. However, how early this occurs and whether it drives tumor development is unclear.

3.1 Prognostic role of checkpoints in breast cancer

With one million new cases in the world each year, breast cancer is one of the most common malignancies (about 24% of all cancer cases), the most common malignancy in women (10–12 per 100 women), and the leading cause of worldwide death for women 40–55 years of age (Baselga and Mendelsohn, 1994; Bange et al., 2001; Chopin et al., 2004a). The pathology is highly resistant to chemotherapy, and there is still no effective cure for the patients with advanced stages of breast cancer, especially in the case of hormone-independent disease (Bange et al., 2001; Chopin et al., 2004a). Most breast cancer patients succumb to the tumor due to the presence of undetected micrometastases; therefore, long-time survival is possible if the tumor becomes localized before metastasing occurs. Since the surgical removal of the primary tumor at very early stages is still the most effective treatment of breast cancer, an early diagnosis of the disease, therefore, would prevent the extremely high morbidity and mortality observed for breast cancer patients. Despite years of intense study, no molecular alterations common to the vast majority of breast cancer have been found till 2000, when Ferguson et al. has observed a silencing 14-3-3 sigma checkpoint gene, the DNA sequence of which is hypermethylated in more than 90% of primary breast cancers tested (Ferguson et al., 2000). This checkpoint is responsible for instituting the cell cycle arrest in G_2 phase (Hermeking et al., 1997; Chan et al., 1999).

The mammalian family of 14-3-3 proteins consists of 7 members, encoded by separate genes (β, γ, ε, η, σ, τ, and ζ) with broad spectrum of functions in kinases conducted signaling pathways generally essential in the controlling of cell cycle progression, particularly during embryonic eye and brain development, as well as regulation of brain function, and general stress response (Aitken et al., 1995; Rittinger et al., 1999). 14-3-3 proteins possess a CpG-rich promoter sequence – the target for a regulation activity of DNA methyltransferases, which control the expression of 14-3-3 proteins on the basic level, by altering their gene methylation status. CpG island methylation is an epigenetic change that is largely responsible for silencing of the corresponding genes. In the case of 14-3-3 checkpoint genes, their hypermethylation may underlie an increased resistance of breast cancer towards genotoxic agents and may lead to a failure of chemo/radio therapies

applied. Some other epigenetic alterations have been demonstrated in breast cancer (50% of breast tumors exhibit a hypermethylation of E-cadherin and mammary derived growth factor inhibitor (Graff et al., 1995; Huynh et al., 1996)), which, however, are not as ubiquitous as it is in the case of the 14-3-3 sigma gene, indicating the importance of this checkpoint loss in breast cancer pathology. Also further checkpoints such as $P21^{WAF1/CIP1}$ and $P27^{KIP1}$ become considered as potent molecular targets for breast cancer therapy (Bange et al., 2001; Chopin et al., 2004a, b).

Molecular mechanisms of the epigenetic control over the overall gene expression have been studied in more detail for the CHFR gene, encoding a mitotic stress checkpoint protein inactivated in plenty of human tumors such as primary colorectal, bone, head and neck cancers (Corn et al., 2003; Toyota et al., 2003). Expression of CHFR correlated well with the methylation status of the gene's CpG-rich regulatory region. The gene hypermethylation and thus silencing of CHFR were strictly dependent on the activities of two DNA methyltransferases, DNMT1 and DNMT3b, inactivation of which restored CHFR expression in tumor cells. Taken together, these findings highlighted the key mechanism by which mitotic checkpoints can be bypassed in cancer.

3.2 Checkpoints in malignant gliomas: "disease proteomics" reveals a failure of current therapy approaches

Human gliomas are the most frequent malignant brain tumors and the most malignant of the astrocytomas. A catastrophic prognosis of the median survival in the range of one year can be only marginally improved by current therapy approaches (Stupp et al., 2005; Wild-Bode et al., 2001). The excessive proliferation, disseminated tumor growth, extremely rich neovascularization, and resistance towards apoptotic stimuli are the main features of malignant gliomas making their treatment especially complicated. The resistance of malignant glioma cells towards stress conditions results in sub-lethal effects under clinically relevant therapy approaches and, consequently, in an enhanced invasiveness of treated cells as revealed by ''Disease proteomics'' (Trog et al., 2006a, b). This scenario cannot lead to success in the therapy of malignant gliomas. In order to reconsider the current therapy approaches, some key mechanisms underlying the response of glioma cells to treatment conditions should be, further, clarified. The clue is obviously a complex molecular and resulting cellular response to sub-lethal genotoxic conditions. Recently achieved results indicate that not a lack of expression, but the deficits in response to genotoxic conditions in terms of P53 and $P21^{WAF1/CIP1}$ expression regulation may play a crucial role in the resistance towards pro-apoptotic stimuli, uncontrolled cell growth and survival of malignant gliomas under clinically relevant chemo- or/and radiation-treatment. In contrast, both P53 and $P21^{WAF1/CIP1}$ were found to be significantly upregulated by high cell density at prolonged cultivation. The well-known specific feature of malignant gliomas is an obligatory dependence on the development of its own vascular system for adequate oxygen and nutrient delivery. Thus, malignant glioma cells of high density induce their own vasculature formation (Ader et al., 2003). It will be speculated, therefore, that the cell cycle control in glioma cells might have a function, which differs from maintaining the original phenotype of single cells: cell cycle attributing proteins are activated in concert with pro-angiogenic events, when the tissue becomes remodeled and the processes of neovascularization are triggered (Trog et al., 2006c). This study gives us an example for the application of checkpoints expression monitoring, in order to evaluate the cancer therapy efficiency and, if necessary, to suggest improved approaches.

4. The key role of checkpoints in longevity/aging and neurodegeneration

4.1 Longevity and accelerated aging

This is one of the phenomena which being associated with organismal stress factors and cellular stress response becomes mostly discussed in literature. Plenty of studies have demonstrated that aging is a result of a complex interaction of genetic, epigenetic, and environmental factors. ''The genetics of human longevity'' undergoes currently an extensive investigation as reviewed by Capri et al. (2006). Different strategies have been proposed which should identify the gene-candidates contributing to either human longevity or aging. Thus, there will be looked for gender differences, since women live longer than men (Austad, 2006), and for a positive association with ''unsuccessful aging'' by selection of genes possibly responsible for myocardial infarction, Alzheimer disease, and *Diabetes mellitus*, etc. (Capri et al., 2006). In this pool of candidates some inflammation-related and oxidative-stress-responsible genes are reported. Further, experiments carried out on long-lived organisms have proposed the decreased cellular production of reactive oxygen species (ROS) in parallel to an increased creation

of antioxidants as the most optimal organismal strategy for longevity (Lutz et al., 2003; Corona et al., 2005). Concretely in mammalians, methionine (Met) metabolism has been shown to be strongly associated with a long life-span (Sanz et al., 2006):

- dietary Met-restriction and shortening of protein Met-content increases the longevity, since this amino acid is most susceptible to oxidation by ROS, and dietary Met-supplementation increases plasma Hcys – an independent risk factor for cardio-vascular disorders as reviewed above,
- knocking out methionine-sulfoxide-reductase (MSR) lowers longevity, whereas its over-expression increases life-span and delays aging (Moskovitz et al., 2001); MSR reduces Met-sulfoxide – the main by-product of protein oxidation – back to Met using a reduction potential of the thioredoxin (TRX)-complex (Stadtman et al., 2003),
- overexpression of thioredoxin controls oxidative stress and life-span increasing considerably the longevity (Mitsui et al., 2002).

Thereby, TRX, an ancient low molecular weight oxido-reductase with highly conservative structure, acts in response to changes in cellular redox status as universal red-ox signaling transductor by ''ping–pong'' mechanism, and represents a functional link to the regulation of hormonal stress processing, DNA metabolism, replication and repair machinery, apoptosis and cell cycle checkpoints (Muller, 1991; Golubnitchaya-Labudova et al., 1998; Turunen et al., 2004; Holmgren et al., 2005). Concerning the checkpoint regulation, recent evidence indicates direct control of the Cdc25s, and therefore the cell cycle, in response to changes in cellular redox status conducted by TRX (Rudolph, 2005).

4.2 Protein redox status, cell cycle control, senescence and neurodegenerative diseases

Oxidative damage to proteins, altered protein redox status, particularly of the key redox systems such as the thioredoxin-dependent methionine-sulfoxide-reductase and the thioredoxin-system, have been implicated in the pathology of several neurodegenerative conditions including Down Syndrome (DS), Alzheimer (AD) and Parkinson diseases (PD), and amyotrophic lateral sclerosis (Kim et al., 2001; Stadtman et al., 2003; Landino et al., 2004). Protein redox status is decisive for the signal transduction targeted in the control over cell cycle progression. The key redox-sensitive cell cycle regulators P53, NFkappaB and the TRX-system represent the functional link between a redox signal cascade and cell cycle machinery (Hinz et al., 1999; Rudolph, 2005; Muller, 1995). A deprivation of both NFkappaB and TRX has been demonstrated early during embryonic development in fetal brain of individuals with DS characterized by neurodegeneration, premature aging, early development of AD, and by an increased risk for cardiovascular and cancer diseases (Labudova et al., 1999b; Kitzmueller et al., 1999). Further analysis revealed strong neuroprotective effects of both NFkappaB and TRX indicating a functional link between altered protein redox status, cell cycle control, and neurodegenerative pathology of both DS and AD (Lovell et al., 2000; Piccioli et al., 2001; Chiueh et al., 2003). A growing body of evidence indicates that neurodegeneration involves the activation of cell cycle machinery in post-mitotic neurons: an accumulation of mitotic phospho-epitopes, reactivation of the M-phase regulator, Cdc2/cyclin B-complex, an activation of Cdc-2-activating Cdc25A-phosphatase as well as modulation of checkpoints activity in G_1/S have been described in neurones undergoing degeneration in AD (Ding et al., 2000; Lim and Qi, 2003). Misregulation of Cdks occurs in AD, PD, amyotrophic lateral sclerosis, and other neurological disorders affecting the following processes: proliferation, differentiation, senescence and apoptosis as reviewed by Nguyen et al. (2002).

Deregulation of cell-cycle-related Cdk2, Cgk4 and Cdk6 initiates death pathways by depressing E2F-1/Rb-dependent transcription at the neuronal G_1/S checkpoints, that, in concert with the activity of cell-cycle-unrelated Cdk5 possibly inducing the cell-cycle proteins, leads to neurodegeneration (Nguyen et al., 2002). Recent experimental data indicate, however, that only stress leading to DNA damage can initiate cell cycle activation linked to neuronal death; in contrast, apoptosis triggered by stimuli that do not target DNA, does not initiate cell cycle activation (Kruman et al., 2004). Therefore, genotoxic stress combined with misregulation of neuronal checkpoints seems to be the key molecular evens underlying neurodegenerative pathology. Inborn checkpoint misregulation is most extensively studied in ATM-deficiency which is accompanied with neuronal degeneration, premature aging and cancer predisposition in *Ataxia telangiectasia*. There is a growing body of evidence that a misregulation of other checkpoints can result in accelerated aging and senescence. Thus, recent animal experiments support the concept that hyper-activation of the checkpoint and tumor suppressor gene p53 may cause accelerated aging (Varela et al., 2005).

Whereas the function of checkpoint genes is relatively well understood in cell cycle progression, little is known about a role of checkpoint genes in non-proliferating cells. As mentioned above, the dysregulated cell cycle activation under genotoxic conditions in non-proliferating cells might be the clue to neurodegeneration and accelerated aging. Thus, apart from the function of universal CDKs inhibitor originally described for $p21^{WAF1/CIP1}$, other functions, particularly, under stress conditions have been demonstrated for this gene as reviewed by Weiss (2003): an induced $p21^{WAF1/CIP1}$ up-regulates a big set of genes implicated in senescence, age-related and neurodegenerative diseases. To this set belongs the β-amyloid precursor protein linked to Alzheimer disease, extracellular matrix components and receptors, cathepsin B, prosaposin, etc. (Chang et al., 2000). Compared to age-matched controls, the $p21^{WAF1/CIP1}$ gene was shown to be hypermethylated in circulating leukocytes of glaucoma patients – a neurodegenerative disease characterized by retinal ganglion cell loss and optic nerve head atrophy (Golubnitschaja et al., 2006c). At the moment we can only speculate about the role of $p21^{WAF1/CIP1}$ hypermethylation in glaucoma, whether this is due to anti-aging processes that act against oxidative stress damage implicated in the pathology of glaucoma or due to a dysregulated epigenetic control initiating cell cycle activation linked to cellular death under genotoxic conditions, which are well documented in glaucoma (Moenkemann et al., 2005). However, a possible dysregulation of epigenetic control over checkpoints expression should be, further, taken into account, if early diagnosis of neurodegenerative diseases and development of new targets for novel therapeutic approaches are considered.

Outlook

There is no doubt about the key role of cell cycle control mechanisms in maintenance of genomic integrity that is absolutely decisive for adequate cellular stress response and appropriate tissue functionality. Inborn genetic checkpoint defects are usually well-detectable through morphological abnormalities like growth and mental retardation, facial dysmorphism, premature aging as well as immune dysfunction, etc., commonly found in human syndromes associated with an impaired response to DNA damage. Predicting a non-inborn tissue specific checkpoint dysregulation associated with a predisposition to tissue specific pathologies such as neurodegeneration, diverse cardiovascular and tumor diseases, is more complicated. However, how early this occurs and whether it drives either tumor development or other pathologies is unclear. Therefore, novel diagnostic approaches should be developed, in order to estimate individual predispositions as early as possible. Molecular research technologies such as ''Disease Proteomics and Transcriptomics'' represent the most powerful tools allowing a precise selection of disease-relevant gene-candidates that can be used as disease-specific molecular markers. Depending on the level of dysregulation an appropriate diagnostic detection method can be chosen such as ''Real-Time''-PCR for the level of transcription, and ''methylation-specific PCR'' for evaluation of epigenetic control quality. One of the most important aspects is the tissue specificity and accessibility. Some non-invasive molecular diagnostic approaches have been recently suggested for early diagnosis of glaucoma and diabetic retinopathy – two neurodegenerative eye disorders with a vascular component in the corresponding pathomechanisms involving pronounced checkpoints dysfunction. Both non-invasive diagnostic approaches are based on disease specific alterations in gene expression profiles of circulating leukocytes that can be evaluated ex vivo in individual blood samples (Golubnitschaja and Flammer, 2004), International Patent No. IB02/00648; Golubnitschaja (2006), International Patent No. 06115230.2). Application of biotechnology in medicine furthers our knowledge about molecular pathomechanisms and opens new perspectives for early diagnosis and even prevention of severe and fatal diseases.

References

Ader I, Delmas C, Bonnet J, Rochaix P, Favre G, Toulas C, Cohen-Jonathan-Moyal E (2003) Inhibition of Rho pathways induces radiosensitization and oxygenation in human glioblastoma xenografts. Oncogene 22: 8861–8869

Aitken A, Jones D, Soneji Y, Howell S (1995) 14-3-3 proteins: biological function and domain structure. Biochem Soc Trans 23: 605–611

Akli S, Zhan S, Abdellatif M, Schneider MD (1999) E1A can provoke G1 exit that is refractory to p21 and independent of activating cdk2. Circ Res 85: 319–328

Akoglu B, Milovic V, Caspary WF, Faust D (2004) Hyperproliferation of homocysteine-treated colon cancer cells is reversed by folate and 5-methyltetrahydrofolate. Eur J Nutr 43: 93–99

Alderton GK, Joenje H, Varon R, Borglum AD, Jeggo PA, O'Driscoll M (2004) Seckel syndrome exhibits cellular features demonstrating defects in the ATR-signalling pathway. Hum Mol Genet 13: 3127–3138

Allan LA, Duhig T, Read M, Fried M (2000) The p21(WAF1/CIP1) promoter is methylated in Rat-1 cells: stable restoration of p53-dependent p21(WAF1/CIP1) expression after transfection of a genomic clone containing the p21(WAF1/CIP1) gene. Mol Cell Biol 20: 1291–1298

Austad SN (2006) Why women live longer than men: sex differences in longevity. Gend Med 3: 79–92

Azuma J, Sawamura A, Awata N (1992) Usefulness of taurine in chronic congestive heart failure and its prospective application. Jpn Circ J 56: 95–99

Bakkenist CJ, Kastan MB (2004) Initiating cellular stress responses. Cell 118: 9–17

Bange J, Zwick E, Ullrich A (2001) Molecular targets for breast cancer therapy and prevention. Nat Med 7: 548–552

Bar MDH, Kreuzer J, Cojoc A, Jahn L (2003) Upregulation of embryonic transcription factors in right ventricular hypertrophy. Basic Res Cardiol 98: 285–294

Baselga J, Mendelsohn J (1994) The epidermal growth factor receptor as a target for therapy in breast carcinoma. Breast Cancer Res Treat 29: 127–138

Boers GH, Smals AG, Trijbels FJ, Fowler B, Bakkeren JA, Schoonderwaldt HC, Kleijer WJ, Kloppenborg PW (1985) Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. N Engl J Med 313: 709–715

Brattstrom LE, Hardebo JE, Hultberg BL (1984) Moderate homocysteinemia – a possible risk factor for arteriosclerotic cerebrovascular disease. Stroke 15: 1012–1016

Brown KD, Rathi A, Kamath R, Beardsley DI, Zhan Q, Mannino JL, Baskaran R (2003) The mismatch repair system is required for S-phase checkpoint activation. Nat Genet 33: 80–84

Buemi M, Marino D, Di Pasquale G, Floccari F, Ruello A, Aloisi C, Corica F, Senatore M, Romeo A, Frisina N (2001) Effects of homocysteine on proliferation, necrosis, and apoptosis of vascular smooth muscle cells in culture and influence of folic acid. Thromb Res 104: 207–213

Burton PB, Yacoub MH, Barton PJ (1999) Cyclin-dependent kinase inhibitor expression in human heart failure. A comparison with fetal development. Eur Heart J 20: 604–611

Capri M, Salvioli S, Sevini F, Valensin S, Celani L, Monti D, Pawelec G, De Benedictis G, Gonos ES, Franceschi C (2006) The genetics of human longevity. Ann NY Acad Sci 1067: 252–263

Carmody BJ, Arora S, Avena R, Cosby K, Sidawy AN (1999) Folic acid inhibits homocysteine-induced proliferation of human arterial smooth muscle cells. J Vasc Surg 30: 1121–1128

Castro R, Rivera I, Struys EA, Jansen EE, Ravasco P, Camilo ME, Blom HJ, Jakobs C, Tavares de Almeida I (2003) Increased homocysteine and S-adenosylhomocysteine concentrations and DNA hypomethylation in vascular disease. Clin Chem 49: 1292–1296

Chan TA, Hermeking H, Lengauer C, Kinzler KW, Vogelstein B (1999) 14-3-3 Sigma is required to prevent mitotic catastrophe after DNA damage. Nature 401: 616–620

Chang BD, Watanabe K, Broude EV, Fang J, Poole JC, Kalinichenko TV, Roninson IB (2000) Effects of p21Waf1/Cip1/Sdi1 on cellular gene expression: implications for carcinogenesis, senescence, and age-related diseases. Proc Natl Acad Sci USA 97: 4291–4296

Charmandari E, Tsigos C, Chrousos G (2005) Endocrinology of the stress response. Annu Rev Physiol 67: 259–284

Chaussalet M, Lamy E, Foucault-Bertaud A, Genovesio C, Sabatier F, Dignat-George F, Charpiot P (2004) Homocysteine modulates the proteolytic potential of human vascular endothelial cells. Biochem Biophys Res Commun 316: 170–176

Chen C, Halkos ME, Surowiec SM, Conklin BS, Lin PH, Lumsden AB (2000) Effects of homocysteine on smooth muscle cell proliferation in both cell culture and artery perfusion culture models. J Surg Res 88: 26–33

Chern CL, Huang RF, Chen YH, Cheng JT, Liu TZ (2001) Folate deficiency-induced oxidative stress and apoptosis are mediated via homocysteine-dependent overproduction of hydrogen peroxide and enhanced activation of NF-kappaB in human Hep G2 cells. Biomed Pharmacother 55: 434–442

Cherubini A, Ruggiero C, Polidori MC, Mecocci P (2005) Potential markers of oxidative stress in stroke. Free Radic Biol Med 39: 841–852

Chiueh C, Lee S, Andoh T, Murphy D (2003) Induction of antioxidative and antiapoptotic thioredoxin supports neuroprotective hypothesis of estrogen. Endocrine 21: 27–31

Chopin V, Toillon RA, Jouy N, Le Bourhis X (2004a) P21(WAF1/CIP1) is dispensable for G1 arrest, but indispensable for apoptosis induced by sodium butyrate in MCF-7 breast cancer cells. Oncogene 23: 21–29

Chopin V, Slomianny C, Hondermarck H, Le Bourhis X (2004b) Synergistic induction of apoptosis in breast cancer cells by cotreatment with butyrate and TNF-alpha, TRAIL, or anti-Fas agonist antibody involves enhancement of death receptors' signaling and requires P21(waf1). Exp Cell Res 298: 560–573

Chuang DM (2005) The antiapoptotic actions of mood stabilizers: molecular mechanisms and therapeutic potentials. Ann NY Acad Sci 1053: 195–204

Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, Graham I (1991) Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 324: 1149–1155

Corn PG, Summers MK, Fogt F, Virmani AK, Gazdar AF, Halazonetis TD, El-Deiry WS (2003) Frequent hypermethylation of the 5′ CpG island of the mitotic stress checkpoint gene Chfr in colorectal and non-small cell lung cancer. Carcinogenesis 24: 47–51

Corona M, Hughes KA, Weaver DB, Robinson GE (2005) Gene expression patterns associated with queen honey bee longevity. Mech Ageing Dev 126: 1230–1238

Coull BM, Malinow MR, Beamer N, Sexton G, Nordt F, de Garmo P (1990) Elevated plasma homocyst(e)ine concentration as a possible independent risk factor for stroke. Stroke 21: 572–576

Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, Nicotera T (1996) Oxidative damage to DNA in diabetes mellitus. Lancet 347: 444–445

Dell'Anna E, Chen Y, Engidawork E, Andersson K, Lubec G, Luthman J, Herrera-Marschitz M (1997) Delayed neuronal death following perinatal asphyxia in rat. Exp Brain Res 115: 105–115

Didenko VV, Ngo H, Minchew CL, Boudreaux DJ, Widmayer MA, Baskin DS (2002) Visualization of irreparable ischemic damage in brain by selective labeling of double strand blunt-ended DNA breaks. Mol Med 8: 818–823

Ding XL, Husseman J, Tomashevski A, Nochlin D, Jin LW, Vincent I (2000) The cell cycle Cdc25A tyrosine phosphatase is activated in degenerating postmitotic neurons in Alzheimer's disease. Am J Pathol 157: 1983–1990

Ferguson AT, Evron E, Umbricht CB, Pandita TK, Chan TA, Hermeking H, Marks JR, Lambers AR, Futreal PA, Stampfer MR, Sukumar S (2000) High frequency of hypermethylation at the 14-3-3 sigma locus leads to gene silencing in breast cancer. Proc Natl Acad Sci USA 97: 6049–6054

Frangogiannis NG, Smith CW, Entman ML (2002) The inflammatory response in myocardial infarction. Cardiovasc Res 53: 31–47

Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuve LP, Rozen R (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 10: 111–113

Golubnitchaya-Labudova O, Horecka T, Kapalla M, Perecko D, Kutejova E, Lubec G (1998) Thioredoxin from Streptomyces aureofaciens controls coiling of plasmid DNA. Life Sci 62: 397–412

Golubnitschaja O, Mönkemann H, Kim K, Mozaffari MS (2003) Depletion of taurine induces DNA damage and expression of checkpoint genes $p21^{WAF1/CIP1}$ and 14-3-3 σ in rat cardiomyocytes. Biochem Pharmacol 66: 511–517

Golubnitschaja O, Flammer J (2004) International Patent No. IB02/00648: Diagnostic method for glaucoma

Golubnitschaja O (2006) International Patent No. 06115230.2: Method for early diagnosis of proliferative diabetic retinopathy

Golubnitschaja O, Moenkemann H, Trog DB, Blom HJ, De Vriese AS (2006a) Activation of genes inducing cell-cycle arrest and of increased DNA repair in the hearts of rats with early streptozotocin-induced diabetes mellitus. Med Sci Monit 12: BR68–BR74

Golubnitschaja O, Yeghiazaryan K, Skowasch D, Schild H, Bauriedel G (2006b) p21(WAF1/CIP1) and 14-3-3 sigma gene expression in degenerated aortic valves: a link between cell cycle checkpoints and calcification. Amino Acids 31: 309–316

Golubnitschaja O, Wunderlich K, Yeghiazaryan K, Schoetzau A, Flammer J (2006c) Hypermethylation of $p21^{WAF1/CIP1}$ and 14-3-3 sigma in leukocytes of glaucoma patients: epigenetic control over senescence progression in stress? Mol Vis (accepted)

Graff JR, Herman JG, Lapidus RG, Chopra H, Xu R, Jarrard DF, Isaacs WB, Pitha PM, Davidson NE, Baylin SB (1995) E-cadherin expression is silenced by DNA hypermethylation in human breast and prostate carcinomas. Cancer Res 55: 5195–5199

Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, Palma-Reis RJ, Boers GH, Sheahan RG, Israelsson B, Uiterwaal CS, Meleady R, McMaster D, Verhoef P, Witteman J, Rubba P, Bellet H, Wautrecht JC, de Valk HW, Sales Luis AC, Parrot-Rouland FM, Tan KS, Higgins I, Garcon D, Andria G, et al. (1997) Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. J Am Med Assoc 277: 1775–1781

Gurusamy N, Watanabe K, Ma M, Zhang S, Muslin AJ, Kodama M, Aizawa Y (2005) Inactivation of 14-3-3 protein exacerbates cardiac hypertrophy and fibrosis through enhanced expression of protein kinase C beta 2 in experimental diabetes. Biol Pharm Bull 28: 957–962

Hartwell LH, Kastan MB (1994) Cell cycle control and cancer. Science 266: 1821–1828

Hegde ML, Gupta VB, Anitha M, Harikrishna T, Shankar SK, Muthane U, Subba Rao K, Jagannatha Rao KS (2006) Studies on genomic DNA topology and stability in brain regions of Parkinson's disease. Arch Biochem Biophys 449: 143–156

Hermeking H, Lengauer C, Polyak K, He TC, Zhang L, Thiagalingam S, Kinzler KW, Vogelstein B (1997) 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. Mol Cell 1: 3–11

Hinokio Y, Suzuki S, Hirai M, Chiba M, Hirai A, Toyota T (1999) Oxidative DNA damage in diabetes mellitus: its association with diabetic complications. Diabetologia 42: 995–998

Hinz M, Krappmann D, Eichten A, Heder A, Scheidereit C, Strauss M (1999) NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. Mol Cell Biol 19: 2690–2698

Holmgren A, Johansson C, Berndt C, Lonn ME, Hudemann C, Lillig CH (2005) Thiol redox control via thioredoxin and glutaredoxin systems. Biochem Soc Trans 33: 1375–1377

Hultberg B, Andersson A, Isaksson A (2000) Hypomethylation as a cause of homocysteine-induced cell damage in human cell lines. Toxicology 147: 69–75

Huynh H, Alpert L, Pollak M (1996) Silencing of the mammary-derived growth inhibitor (MDGI) gene in breast neoplasms is associated with epigenetic changes. Cancer Res 56: 4865–4870

Irie M, Asami S, Nagata S, Ikeda M, Miyata M, Kasai H (2001) Psychosocial factors as a potential trigger of oxidative DNA damage in human leukocytes. Jpn J Cancer Res 92: 367–376

Kartal Ozer N, Taha S, Azzi A (2005) Homocysteine induces DNA synthesis and proliferation of vascular smooth muscle cells by interfering with MAPK kinase pathway. Biofactors 24: 193–199

Kawabe T (2004) G2 checkpoint abrogators as anticancer drugs. Mol Cancer Ther 3: 513–519

Kim SH, Fountoulakis M, Cairns N, Lubec G (2001) Protein levels of human peroxiredoxin subtypes in brains of patients with Alzheimer's disease and Down syndrome. J Neural Transm [Suppl] 61: 223–235

Kitzmueller E, Labudova O, Rink H, Cairns N, Lubec G (1999) Altered gene expression in fetal Down syndrome brain as revealed by the gene hunting technique of subtractive hybridization. J Neural Transm [Suppl] 57: 99–124

Korystov IuN (1997) Emotions, stress, smoking, alcohol consumption and cancer – the correlational and causal connections. Zh Vyssh Nerv Deiat Im I P Pavlova 47: 627–657

Krauss H, Kozlik J, Grzymislawski M, Sosnowski P, Mikrut K, Piatek J, Paluszak J (2003) The influence of glimepiride on the oxidative state of rats with streptozotocin-induced hyperglycemia. Med Sci Monit 9: 389–393

Kruman II, Wersto RP, Cardozo-Pelaez F, Smilenov L, Chan SL, Chrest FJ, Emokpae R Jr, Gorospe M, Mattson MP (2004) Cell cycle activation linked to neuronal cell death initiated by DNA damage. Neuron 41: 549–561

Kultz D (2003) Evolution of the cellular stress proteome: from monophyletic origin to ubiquitous function. J Exp Biol 206: 3119–3124

Kultz D (2005) Molecular and evolutionary basis of the cellular stress response. Annu Rev Physiol 67: 225–257

Labudova O, Yeghiazarjan C, Hoger H, Lubec G (1999a) Taurine modulates expression of transporters in rat brain and heart. Amino Acids 17: 301–313

Labudova O, Kitzmueller E, Rink H, Cairns N, Lubec G (1999b) Gene expression in fetal Down syndrome brain as revealed by subtractive hybridization. J Neural Transm [Suppl] 57: 125–136

Landino LM, Skreslet TE, Alston JA (2004) Cysteine oxidation of tau and microtubule-associated protein-2 by peroxynitrite: modulation of microtubule assembly kinetics by the thioredoxin reductase system. J Biol Chem 279: 35101–35105

Leinonen J, Lehtimaki T, Toyokuni S, Okada K, Tanaka T, Hiai H, Ochi H, Laippala P, Rantalaiho V, Wirta O, Pasternack A, Alho H (1997) New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. FEBS Lett 417: 150–152

Levitt PS, Liu H, Manning C, Weiss RS (2005) Conditional inactivation of the mouse Hus1 cell cycle checkpoint gene. Genomics 86: 212–224

Lewis JD, Meehan RR, Henzel WJ, Maurer-Fogy I, Jeppesen P, Klein F, Bird A (1992) Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. Cell 69: 905–914

Li J, Chai S, Tang C, Du J (2003) Homocysteine potentiates calcification of cultured rat aortic smooth muscle cells. Life Sci 74: 451–461

Liang Q, Carlson EC, Donthi RV, Kralik PM, Shen X, Epstein PN (2002) Overexpression of metallothionein reduces diabetic cardiomyopathy. Diabetes 51: 174–181

Lim AC, Qi RZ (2003) Cyclin-dependent kinases in neural development and degeneration. J Alzheimers Dis 5: 329–335

Limana F, Urbanek K, Chimenti S, Quaini F, Leri A, Kajstura J, Nadal-Ginard B, Izumo S, Anversa P (2002) bcl-2 overexpression promotes myocyte proliferation. Proc Natl Acad Sci USA 99: 6257–6262

Liu ZP, Olson EN (2002) Suppression of proliferation and cardiomyocyte hypertrophy by CHAMP, a cardiac-specific RNA helicase. Proc Natl Acad Sci USA 99: 2043–2048

Lovell MA, Xie C, Gabbita SP, Markesbery WR (2000) Decreased thioredoxin and increased thioredoxin reductase levels in Alzheimer's disease brain. Free Radic Biol Med 28: 418–427

Lubec B, Szekeres T (1994) Growth promotion by homocysteine thiolactone and its alpha-alkylated derivative. Amino Acids 7: 109–111

Lubec B, Labudova O, Hoeger H, Muehl A, Fang-Kircher S, Marx M, Mosgoeller W, Gialamas J (1996) Homocysteine increases cyclin-dependent kinase in aortic rat tissue. Circulation 94: 2620–2625

Lutgens E, de Muinck ED, Kitslaar PJ, Tordoir JH, Wellens HJ, Daemen MJ (1999) Biphasic pattern of cell turnover characterizes the progression from fatty streaks to ruptured human atherosclerotic plaques. Cardiovasc Res 41: 473–479

Lutz PL, Prentice HM, Milton SL (2003) Is turtle longevity linked to enhanced mechanisms for surviving brain anoxia and reoxygenation? Exp Gerontol 38: 797–800

Malinow MR, Kang SS, Taylor LM, Wong PW, Coull B, Inahara T, Mukerjee D, Sexton G, Upson B (1989) Prevalence of hyperhomocyst(e)inemia in patients with peripheral arterial occlusive disease. Circulation 79: 1180–1188

Malinow MR, Ducimetiere P, Luc G, Evans AE, Arveiler D, Cambien F, Upson BM (1996) Plasma homocyst(e)ine levels and graded risk for myocardial infarction: findings in two populations at contrasting risk for coronary heart disease. Atherosclerosis 126: 27–34

Mann MJ, Gibbons GH, Tsao PS, von der Leyen HE, Cooke JP, Buitrago R, Kernoff R, Dzau VJ (1997) Cell cycle inhibition preserves endothelial function in genetically engineered rabbit vein grafts. J Clin Invest 99: 1295–1301

Massague J (2004) G1 cell-cycle control and cancer. Nature 432: 298–306

McGowan CH, Russell P (2004) The DNA damage response: sensing and signaling. Curr Opin Cell Biol 16: 629–633

Mercier I, Colombo F, Mader S, Calderone A (2002) Ovarian hormones induce TGF-beta(3) and fibronectin mRNAs but exhibit a disparate action on cardiac fibroblast proliferation. Cardiovasc Res 53: 728–739

Midorikawa K, Kawanishi S (2001) Superoxide dismutases enhance H_2O_2-induced DNA damage and alter its site specificity. FEBS Lett 495: 187–190

Migliore L, Fontana I, Trippi F, Colognato R, Coppede F, Tognoni G, Nucciarone B, Siciliano G (2005) Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. Neurobiol Aging 26: 567–573

Mitsui A, Hamuro J, Nakamura H, Kondo N, Hirabayashi Y, Ishizaki-Koizumi S, Hirakawa T, Inoue T, Yodoi J (2002) Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span. Antioxid Redox Signal 4: 693–696

Moenkemann H, Labudova O, Yeghiazarian K, Rink H, Hoeger H, Lubec G (1999) Evidence that taurine modulates osmoregulation by modification of osmolarity sensor protein ENVZ-expression. Amino Acids 17: 347–355

Moenkemann H, De Vriese AS, Blom HJ, Kluijtmans LA, Heil SG, Schild HH, Golubnitschaja O (2002) Early molecular events in the development of the diabetic cardiomyopathy. Amino Acids 23: 331–336

Moenkemann H, Flammer J, Wunderlich K, Breipohl W, Schild HH, Golubnitschaja O (2005) Increased DNA breaks and up-regulation of both G(1) and G(2) checkpoint genes p21(WAF1/CIP1) and 14-3-3 sigma in circulating leukocytes of glaucoma patients and vasospastic individuals. Amino Acids 28: 199–205

Moskovitz J, Bar-Noy S, Williams WM, Requena J, Berlett BS, Stadtman ER (2001) Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. Proc Natl Acad Sci USA 98: 12920–12925

Mozaffari MS, Schaffer D (2001) Taurine modulates arginine vasopressin-mediated regulation of renal function. J Cardiovasc Pharmacol 37: 742–750

Muller EG (1991) Thioredoxin deficiency in yeast prolongs S phase and shortens the G1 interval of the cell cycle. J Biol Chem 266: 9194–9202

Muller EG (1995) A redox-dependent function of thioredoxin is necessary to sustain a rapid rate of DNA synthesis in yeast. Arch Biochem Biophys 318: 356–361

Nadal-Ginard B (2001) Generation of new cardiomyocytes in the adult heart: prospects of myocardial regeneration as an alternative to cardiac transplantation. Rev Esp Cardiol 54: 543–550

Nguyen MD, Mushynski WE, Julien JP (2002) Cycling at the interface between neurodevelopment and neurodegeneration. Cell Death Differ 9: 1294–1306

Paulson DJ (1997) The diabetic heart is more sensitive to ischemic injury. Cardiovasc Res 34: 104–112

Pereg Y, Lam S, Teunisse A, Biton S, Meulmeester E, Mittelman L, Buscemi G, Okamoto K, Taya Y, Shiloh Y, Jochemsen AG (2006) Differential roles of ATM- and Chk2-mediated phosphorylations of Hdmx in response to DNA damage. Mol Cell Biol 26: 6819–6831

Piccioli P, Porcile C, Stanzione S, Bisaglia M, Bajetto A, Bonavia R, Florio T, Schettini G (2001) Inhibition of nuclear factor-kappaB activation induces apoptosis in cerebellar granule cells. J Neurosci Res 66: 1064–1073

Pion PD, Kittleson MD, Rogers QR, Morris JG (1987) Myocardial failure in cats associated with low plasma taurine: a reversible cardiomyopathy. Science 237: 764–768

Rittinger K, Budman J, Xu J, Volinia S, Cantley LC, Smerdon SJ, Gamblin SJ, Yaffe MB (1999) Structural analysis of 14-3-3 phosphopeptide complexes identifies a dual role for the nuclear export signal of 14-3-3 in ligand binding. Mol Cell 4: 153–166

Rudolph J (2005) Redox regulation of the Cdc25 phosphatases. Antioxid Redox Signal 7: 761–767

Sachdev PS (2005) Homocysteine and brain atrophy. Prog Neuropsychopharmacol Biol Psychiatry 29: 1152–1161

Sampson MJ, Astley S, Richardson T, Willis G, Davies IR, Hughes DA, Southon S (2001) Increased DNA oxidative susceptibility without increased plasma LDL oxidizability in Type II diabetes: effects of alpha-tocopherol supplementation. Clin Sci (Lond) 101: 235–241

Sanders DB, Hunter K, Wu Y, Jablonowski C, Bahl JJ, Larson DF (2001) Modulation of the inflammatory response in the cardiomyocyte and macrophage. J Extra Corpor Technol 33: 167–174

Sanz A, Caro P, Ayala V, Portero-Otin M, Pamplona R, Barja G (2006) Methionine restriction decreases mitochondrial oxygen radical generation and leak as well as oxidative damage to mitochondrial DNA and proteins. FASEB J 20: 1064–1073

Sardas S, Yilmaz M, Oztok U, Cakir N, Karakaya AE (2001) Assessment of DNA strand breakage by comet assay in diabetic patients and the role of antioxidant supplementation. Mutat Res 490: 123–129

Schaffer S, Solodushko V, Azuma J (2000a) Taurine deficient cardiomyopathy: role of phospholipids, calcium and osmotic stress. Adv Exp Med Biol 483: 57–69

Schaffer SW, Lombardini JB, Azuma J (2000b) Interaction between the actions of taurine and angiotensin II. Amino Acids 18: 305–318

Schmidt HH, Walter U (1994) NO at work. Cell 78: 919–925

Sherr CJ (1994) G1 phase progression: cycling on cue. Cell 79: 551–555

Shiloh Y (2003) ATM and related protein kinases: safeguarding genome integrity. Nat Rev Cancer 3: 155–168

Simmen MW, Leitgeb S, Charlton J, Jones SJ, Harris BR, Clark VH, Bird A (1999) Nonmethylated transposable elements and methylated genes in a chordate genome. Science 283: 1164–1167

Sole MJ, Jeejeebhoy KN (2002) Conditioned nutritional requirements: therapeutic relevance to heart failure. Herz 27: 174–178

Stadtman ER, Moskovitz J, Levine RL (2003) Oxidation of methionine residues of proteins: biological consequences. Antioxid Redox Signal 5: 577–582

Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO (2005) European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352: 987–996

Suleiman MS (1994) New concepts in the cardioprotective action of magnesium and taurine during the calcium paradox and ischaemia of the heart. Magnes Res 7: 295–312

Tamamori M, Ito H, Hiroe M, Terada Y, Marumo F, Ikeda MA (1998) Essential roles for G1 cyclin-dependent kinase activity in development of cardiomyocyte hypertrophy. Am J Physiol 275: H2036–H2040

Taniguchi N, Kaneto H, Asahi M, Takahashi M, Wenyi C, Higashiyama S, Fujii J, Suzuki K, Kayanoki Y (1996) Involvement of glycation and oxidative stress in diabetic macroangiopathy. Diabetes 45: S81–S83

Tate P, Skarnes W, Bird A (1996) The methyl-CpG binding protein MeCP2 is essential for embryonic development in the mouse. Nat Genet 12: 205–208

Tomei LD, Kiecolt-Glaser JK, Kennedy S, Glaser R (1990) Psychological stress and phorbol ester inhibition of radiation-induced apoptosis in human peripheral blood leukocytes. Psychiatry Res 33: 59–71

Tosaki A, Engelman DT, Engelman RM, Das DK (1996) The evolution of diabetic response to ischemia/reperfusion and preconditioning in isolated working rat hearts. Cardiovasc Res 31: 526–536

Toyota M, Sasaki Y, Satoh A, Ogi K, Kikuchi T, Suzuki H, Mita H, Tanaka N, Itoh F, Issa JP, Jair KW, Schuebel KE, Imai K, Tokino T (2003) Epigenetic inactivation of CHFR in human tumors. Proc Natl Acad Sci USA 100: 7818–7823

Trog D, Fountoulakis M, Friedlein A, Golubnitschaja O (2006a) Is current therapy of malignant gliomas beneficial for patients? Proteomics evidence of shifts in glioma cells expression patterns under clinically relevant treatment conditions. Proteomics 6: 2924–2930

Trog D, Yeghiazaryan K, Fountoulakis M, Friedlein A, Moenkemann H, Haertel N, Schueller H, Breipohl W, Schild H, Leppert D, Golubnitschaja O (2006b) Pro-invasive gene regulating effect of irradiation and combined temozolomide-radiation treatment on surviving human malignant glioma cells. Eur J Pharmacol 542: 8–15

Trog D, Moenkemann H, Breipohl W, Schueller H, Schild H, Golubnitschaja O (2006c) Non-sufficient cell cycle control as possible clue for the resistance of human malignant glioma cells to clinically relevant treatment conditions. Amino Acids [Epub ahead of print] PMID: 17077962

Tsai JC, Perrella MA, Yoshizumi M, Hsieh CM, Haber E, Schlegel R, Lee ME (1994) Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. Proc Natl Acad Sci USA 91: 6369–6373

Turunen N, Karihtala P, Mantyniemi A, Sormunen R, Holmgren A, Kinnula VL, Soini Y (2004) Thioredoxin is associated with proliferation, p53 expression and negative estrogen and progesterone receptor status in breast carcinoma. APMIS 112: 123–132

Varela I, Cadinanos J, Pendas AM, Gutierrez-Fernandez A, Folgueras AR, Sanchez LM, Zhou Z, Rodriguez FJ, Stewart CL, Vega JA, Tryggvason K, Freije JM, Lopez-Otin C (2005) Accelerated ageing in mice deficient in Zmpste24 protease is linked to p53 signalling activation. Nature 437: 564–568

von Harsdorf R, Hauck L, Mehrhof F, Wegenka U, Cardoso MC, Dietz R (1999) E2F-1 overexpression in cardiomyocytes induces downregulation of p21CIP1 and p27 KIP1 and release of active cyclin-dependent kinases in the presence of insulin-like growth factor I. Circ Res 85: 128–136

Weiss RH (2003) p21Waf1/Cip1 as a therapeutic target in breast and other cancers. Cancer Cell 4: 425–429

Wilcken DE, Reddy SG, Gupta VJ (1983) Homocysteinemia, ischemic heart disease, and the carrier state for homocystinuria. Metabolism 32: 363–370

Wild-Bode C, Weller M, Rimner A, Dichgans J, Wick W (2001) Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. Cancer Res 61: 2744–2750

Xie G, Habbersett RC, Jia Y, Peterson SR, Lehnert BE, Bradbury EM, D'Anna JA (1998) Requirements for p53 and the ATM gene product in the regulation of G1/S and S phase checkpoints. Oncogene 16: 721–736

Yang ZZ, Zou AP (2003) Homocysteine enhances TIMP-1 expression and cell proliferation associated with NADH oxidase in rat mesangial cells. Kidney Int 63: 1012–1020

Zhang Q, Zeng X, Guo J, Wang X (2001) Effects of homocysteine on murine splenic B lymphocyte proliferation and its signal transduction mechanism. Cardiovasc Res 52: 328–336

Zhang Q, Zeng X, Guo J, Wang X (2002) Oxidant stress mechanism of homocysteine potentiating Con A-induced proliferation in murine splenic T lymphocytes. Cardiovasc Res 53: 1035–1042

Zhuang JC, Wogan GN (1997) Growth and viability of macrophages continuously stimulated to produce nitric oxide. Proc Natl Acad Sci USA 94: 11875–11880

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