Mutagenesis Associated with DNA-Damage-Stress Response

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Abstract: In this manuscript we aim to take on *the controversies in oncology,* such as those *related to costs and screening guidelines.* Are we recently at new turning point with our existing *methodology?* During the past 30 years of evolution methods of *awareness* and *screening* have lead to an emphasis on *early diagnoses of cancer* and not only cancer. Recent *trends* and *clinical trials* show that these goals have *not been met.* International data demonstrate significant increase in *early stage disease, without a proportional decrease* in later-stage disease. We need to *more differentiate in methodology* because cancers are heterogenous with multiple paths, *not all* of which progress to metastases and death. This *broader definition* of disease named cancer must include also *indolent disease* that causes *no* harm during patients lifetime. *Overdiagnosis and overtreatment* result in billions of USD of wasted money. Our methodology must be *completed* with methods which take proper account of the *real-life environment.*

Keywords: The stress-induced mutagenesis, the epigenetic diversity, stress response, awareness and screening, overdiagnosis, overtreatment.

1. INTRODUCTION – THE NEW PARADIGM FOR MUTATION-RATES WITHIN BACTERIA

Basic ideas about the constancy and randomness of *mutagenesis* that drives evolution were challenged by the discovery of mutation pathways *activated by stress responses.* These pathways could promote evolution specifically when cells are *maladapted to their environment* (i.e., *are stressed*). How, when, and where mutations form underpins understanding *pathogen-host interactions, antibiotic resistance, aging, cancer progression therapy resistance, and evolution generally.* Bacterial yeast, human cells appear to possess *mechanisms* that *induce mutation pathways* specifically during stress, under *the control of stress response (stress-induced mutagenesis* or *SIM).* These pathways suggest mechanisms by which *genetic diversity* could be generated preferentially when cells are maladapted to their environment, potentially accelerating evolution responsively to environments, a major departure from classic views.

The strongest support for the idea of *increased mutation rate* during a general stress, and the most detailed *understanding* of a *molecular mechanism,* comes from starving *Escherischia coli* in an assay, the mechanism behind which has been debated. This mechanism appears to entail a *switch* from high-fidelity to *error-prone DNA double-strand break (DSB) repair under stress,* controlled by the SOS DNA-damage and *the RpoS general-stress-response activator/*starvation stress responses.

Each stress response is necessary, but not sufficient for the switch to *mutagenic repair.* Both confer up-regulation of the DinB error-prone DNA polymerase, which then makes errors that *become mutations* in acts of DSB repair via homologous recombination (HR). Whereas up-regulation of DinB is the sole role os SOS in DSB-dependent SIM, RpoS additionally licenses the use of DinB and other low-fidelity DNA polymerases in DSB repair by a mechanism not fully elucidated. This process cauces a switch from high-fidelity to *mutagenic DSB repair* under *RpoS-inducing stress.* RpoS is activated by many *general stressors,* including starvation, osmotic shock, cold shock, and *oxidative stress,* making DSB-dependent SIM potentially important for producing genetic diversity when cells are maladapted to many stressing environments. SOS is induced in most acts of DSB repair, making RpoS the switch to error-prone repair during stress.

Here we show that DSB-dependent SIM occurs in the bacterial chromosome of plasmid-free cells, does not result from growth of selected gene amplifications promoting mutation independently of stress and, moreover, represents about half of spontaneous frame-shift and base- substitution mutagenesis in starving cells. These data indicate the generality of DSB-dependent stress-induced mutation, rule out major alternative models for the mechanism, and define a mechanism underlying a major component of *spontaneous mutation* in *E. coli* [1].

It has been shown that the mutation rate may be increased in response to stress (starvation, antibiotics, and other environmental challenges). Double-strand-break-dependent stress-induced mutagenesis in E. coli occurs when *three events occur simultaneously*: (i) the

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formation of repair by homologous recombination of a DNA double-strand break or double-strand end (DSB/DSE); (ii) induction of the bacterial DNA-damage response, the SOS response, which DSBs/DSEs induce; and (iii) a second stress, unrelated to the DSB/DSE, that activates the general or starvation *stress response controlled* by RpoS (σ^S) transcriptional activator. DSBs/DSEs occur spontaneously in just over 10^{-3} of growing E. coli cells and induce the SOS response about 25% of the time that are repaired. Event number 2, the SOS response, is a consequence of event 1, formation of a DSB [1, 2].

The power of identifying *the proteins* and their mechanisms of action in stress-inducible mutation pathways is, that with their identities comes the potential to *inhibit* these patways *therapeutically.* In the future it may be possible to take *anti-evolution drugs to block stress-promoted adaptation of pathogens* to host-instigated stressors. *New strategies* would include *inhibiting* stress-induced mutation pathways. These *fundamentally different strategies* and antibiotic or antifugal drugs would work by *suppressing pathogen evolution* while *the immune system* catches up.

2. REASONS FOR INTERDISCIPLINARY METHODOLOGY

As it can be seen from *the enigmatic title* of our recent paper, we are interested in *advocacy for holism/interdisciplinary methodology* especially in *cancer research* and *biology.* Our approach is close to the recent *insight hypothesis* offered by Davies & Lineweaver (D&L) which states that *cancers are too organized to be mere random mutations,* but are probably a *reversion to an epigenetic configuration characteristic of (i) embryos, and (ii) genetic forebears* [3].

Along with the above discussions like the D&L hypothesis and questions relating to UV&/or mitochondria, (ii) we are given a central attention to *the causal chain relating stress and mutations* as it can be seen in the work of S. Rosenberg [1, 2].

Recent findings indicate that double-strand break *(DSB)*-repair-dependent stress-induced mutation driven by spontaneous DNA breaks is a *pathway* that cells use as a major source of spontaneous mutation. *Mechanisms* coupling mutagenesis to *stress responses* allow cells to evolve rapidly and responsively to their *environment* [4]. Bacterial and human cells use *mechanisms* that *induce mutation* pathways under *the control of stress responses.*

The stress-induced mutagenesis is an *evolutionary engine* which generates *the epigenetic diversity.* This process causes a switch from high-fidelity to mutagenic DSB repair under RpoS general-stress-response activator-inducing stress. RpoS is activated by general stressors, like starvation, osmotic shock, cold shock, and oxidative stress, making DSB-dependent SIM potentially decissive for producing epigenetic diversity. Furthermore, stress is not required, activation of *the stress response* is *sufficent.* The mutagenesis occurs only in stressed cells and in unstressed growing cells if the RpoS response is activated artificially.

Coupling of inducible mutagenesis pathways to a general stress response as RpoS causes epigenetic diversity generated responsively to different stressors and environments. Stress-induced mutagenesis and phenotypic diversification also contribute to *climate/heat stress-induced* increased expression of epigenetic variation causing rapid evolution [2, 5].

> "Life expectancy for someone with metastatic cancer has hardly changed in five decades, despite all the hype about imminent „cures". It is clear that *some radical new thinking* is needed. ...its effect can certainly be mitigated for example, by delaying onset and extending dormancy. But we will learn to do this effectively only when we *better understand cancer*, including its place in the great sweep of *evolutionary* history" [3].

Thus, DSE-dependent stress-induced mutagenesis is *controlled critically by RpoS,* which *switches* DSE repair from a *high-fidelity mode* using PoIIII to an *error-prone mutagenic process,* using error-prone DNA polymerases when cells are *stressed,* potentially *accelerating evolution* specifically under *stress.*

3. CLINICAL FAILURE – MISTAKEN IDEA ABOUT CAUSAL CHAIN FOR CANCER

The problem is, that recently we are at *new turning point* with our existing *methodology*. During the past 30 years of above evolution methods of *awareness* and *screening* have lead to an emphasis on *early diagnoses of cancer* and not only cancer [6].

It can be hypothesized, that our methodology must be *completed* with methods which take proper account of the *real-life environment.* This approach, often called "*ethology"* is typically used in *epigenetics* and *psychiatry* to reveal *links* between *neural, endocrine,*

immune, social, and *psychological brain function.* It means use more *interdisciplinary effort.* A *multidisciplinary effort* include also *the pathology, imaging, surgical, advocate,* and *medical communities* could be convened by an independent group to *revise the taxonomy of lesions* now called *cancer* and to create *reclassification criteria* for IDLE conditions.

Bacterial and human cells use *mechanisms* that *induce mutation* pathways under *the control of stress responses.* The links between inflammation, metabolic syndrome, and cancer show that distinct diseases can arise from fundamental abberations in *metabolism, homeostasis,* and *immune function. The multidisciplinary effort could use* the advances in *systems biology,* providing evidence for some of *holistic concepts.*

Also *the goals* based on early diagnoses of cancer *not reduced* the level of late-stage disease and *decrease* cancer mortality. Recent *trends* and *clinical trials* show that these goals have *not been met.* International data demonstrate significant increase in *early stage disease, without* a proportional decrease in later-stage disease [6, 7].

For me it means, that we must *re-formulate* the definition of *pathologic condition* of cancer. We need to *more differentiate in methodology* because cancers are heterogenous with multiple paths, *not all* of which progress to metastases and death. This *broader definition* of disease named cancer must include also *indolent disease* that causes *no* harm during patients lifetime. Complexity of possibble forms of cancers means that we must try to work with *more broad frame of its early diagnosis* [8].

Methodological turning-point require to adapt cancer screening to *higher analytical level* of identifying and treating of *epigenetical* and *psychiatric conditions* associated with disease, morbidity and mortality. For example, screening of breast cancer and prostate cancer detect more cases of cancer than are *potentially clinically insignificant.* The detection and lesions removal in breast cancer considered precancerous *freequently not lead to lower* incidence of invasive cancer [6]. *Overdiagnosis and overtreatment* results in billions USD of wasted money.

4. EVIDENCE THAT SOCIAL EVENTS AFFECT GENE EXPRESSION

Diagnosis of the disease can no longer ignore *the social conditions* of the patients, because it is known that *social stress have adverse health effects* on both humans and primates. Aditionally, social stress have a strong and pervasive effect on *the regulation of the genome.* Now researchers found differences in the expression of nearly 1,000 genes in the white blood cells of macaques, which play a role in the immunity. Altough all of the monkeys have the same set of genes in their white blood cells, not all of them turn on these genes to the same degree. We are now able to predict *the social ranking* of a macaque with 80 percent accuracy simply by looking at *the genes* [5, 9, 10].

Gene-environment interactions in humans and nonhuman primates frequently include *allelic variants* that act via *altering gene expression levels.* These evidence lines suggests that *social status* might also *directly influence* gene regulation in primates.

In [5] the researchers adressed questions of variation in the social environment as a fundamental component of many vertebrate societies. In humans and other primates, adverse social environments often *translate* into *lasting physiological costs.* The biological mechanisms associated with these effects are therefore of great interest, for *understanding* of the mechanisms that mediate these effects at *molecular level.* They adressed above questions by leveraging the power of *the experimental system* that consisted of 10 social groups of female macaques, in which each individual's social status (i.e. *dominance rank*) could be experimentally controlled. Using this paradigm, they showed that the dominance rank results in a widespread, yet plastic, *imprint on gene regulation.*

They have investigated the mechanistic basis of these effects using *cell type-specific gene expression profiling* and *glucocorticoid resistance assays,* which together contributed to rank effects on *gene expression levels* for 694 (70%) of the 987 *rank-related genes.*

Paralell effects of *dominance rank* on *social environment* and *gene expression levels* have been confirmed. *Low-ranking* individuals experience *more aggression* from group mates than *high-ranking* individuals ($P<10^{-6}$, $R^2 = 0.42$, $n=49$). This social experience is mirrored by gene expression profiles that vary according to rank. Principal component (PC)1 explains 20,2% of overall variance in gene expression and is correlated with rank ($P=0.03$, $R^2 = 0.10, n=49$). On the heatmaps of $\log_2-$transformed gene expression levels for rank-associated genes. Values have shown after controlling for differences in means among social groups; 0 roughly corresponded to mean expression levels.

Recently is known relationship between dominance rank and another *mechanism* that mediate social environmental effects on the genome, *DNA methylation* [5, 11]. In this connection is important that *peripheral blood mononuclear cell (*PBMC) gene expression data ($CD4^+$, T cells, B cells, $CD8^+$, and monocytes) *alone predict social status* with 80% accuracy. In chronic stress exposure is disregulated *hypothalamic-pituitary adrenal axis* (HPA) axis activity. *DNA methylation is a regulatory mechanism linked to social environmental effects and gene expression* [4, 12, 13].

A strong and widespread association between dominance rank and gene regulation in PBMCs show sensitivity to social environment reflected in changes in gene expression in *the immune system*. There is a *link* between *neural, endocrine* and *immune* function. These results support the idea that changes in gene regulation are given by links between *the social environment* and *physiology.* Potentially they are supplying an important piece to the puzzle of how social effects regulate the human health, for example in the context of *cancer* [7].

Some findings suggest that the timescale for social effects on *epigenetic variations* also *extends to adulthood*. Such effects include the components of social structure, like the dominance rank. For example, *NR3C1,* the GC receptor gene, plays a key role in linking *behavior, HPA axis- mediated stress responses,* and *gene expression* [1, 5]. Psychological stress and exposure to the stressor *social disruption (SDR)* increase cytokine production by monocytes/ macrophages and reduce their sensitivity to corticosterone. Splenic monocytes/macrophages from socially stressed mice are primed to be more physiologically active than nonstressed controls [8, 14].

A new study suggests B cells undergo spontaneous changes that could lead to cancer if *the immune system* does not carry out regular checks and kill them before they form *tumors. Malignant transformation* of mature B cells requires *mutations impairing* intrinsic differentiation processes and *permit escape from T cell-mediated tumor surveillance* [15]. Even growing tumors may be subject to *immunosurveillance* and killing by activated T and NK cells. It is likely that *immunosurveilance* and tumor-promoting inflammation can coexist even in the same tumor [16].

5. PREMATURE DIAGNOSIS – A CLINICAL WORRY, BUT ALREADY KNOWN

The goals based on early diagnoses of cancer not reduced the level of late-stage disease and decrease cancer mortality. Recent *trends* and *clinical trials* show that these goals have *not been met.* International data demonstrate significant increase in *early stage disease,* without a proportional decrease in later-stage disease [6, 17]. The detection and removal of lesions considered precancerous have *not* led to lower incidence of invasive cancer.

6. CONCLUSIONS ON HOW TO VIEW THESE CLINICAL FINDINGS

It can be hypothesized, that our methodology must be *completed* with methods which take proper account of the *real-life environment* [3, 6]. This approach, often called *„ethology“* is typically used in *epigenetics* and *psychiatry* to reveal *links* between *neural, endocrine, immune, social,* and *psychological brain function.* It means use more *interdisciplinary effort.* A *multidisciplinary effort* include also *the pathology, imaging, surgical, advocate,* and *medical communities* could be convened by an independent group to *revise the taxonomy of lesions* now called *cancer* and to create *reclassification criteria* for IDLE conditions.

Bacterial and human cells use *mechanisms* that *induce mutation* pathways under *the control of stress responses.* The links between inflammation, metabolic syndrome, and cancer show that distinct diseases can arise from fundamental abberations in *metabolism, homeostasis,* and *immune function. The multidisciplinary effort could use* the advances in *systems biology,* providing evidence for some of *holistic concepts.*

They may be *a response* to Davies call for “*some radically new thinking“* in cancer therapy because “*we better understand cancer”* [3]. Treatment regimes are largely designed to restore *the potential* of organism’s *natural defense mechanisms* and *self-control powers* regulated by the healing principles of *new complex therapy*. We need to use *more flexible methods* to *sufficent treatment* of short-lived pathological conditions diagnosed as a *cancer* [6]. This old practice is leeding to *misuse of bilions of USD.*

Research must include the mechanisms of *controlling the environment* as an *alternative* to surgical excision which must be *replaced by the less invasive interventions* in whole.

The risk of *overdiagnosis cancer, overthreat, screening* and *patient awareness* must *minimize* by the use of *epigenetic* and *psychiatric analysis* and they

must be included into the methodology of cancer diagnosis as its *stable part*.

We can *renew the methodology of cancer treatment* outgoing from the principle that stress is not required for mutation, *activation of the stress response is sufficient.* Complementary to *the bottom-up* approach, bigger role can play also *down-stream* analysis of a stress-response-controlled *switch* to mutagenic pathways. Future research may improve *brain links* to *the potential* for social regulation of gene expression in the immune system and its influence on the individual physiology.

The molecular mechanisms of genetic and non-genetic inheritance include many yet to be dissected, but are fundamental to detailed and specific *understanding of evolution,* which in turn may bring *realistic strategies* against *evolution-driven problems* like *cancer* and infectious disease [1].

CONFLICT OF INTEREST

The author declares no conflict of interest.

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