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Step-wise and punctuated genome evolution drive phenotype changes of tumor cells

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ABSTRACT

The pattern of genome evolution can be divided into two phases: the step-wise continuous phase (step-wise clonal evolution, stable dominant clonal chromosome aberrations (CCAs), and low frequency of non-CCAs, NCCAs) and punctuated phase (marked by elevated NCCAs and transitional CCAs). Depending on the phase, system stresses (the diverse CIN promoting factors) may lead to the very different phenotype responses. To address the contribution of chromosome instability (CIN) to phenotype changes of tumor cells, we characterized CCAs/NCCAs of HeLa and HEK293 cells, and their derivatives after genotoxic stresses (a stable plasmid transfection, ectopic expression of cancer-associated *CHI3L1* gene or treatment with temozolomide) by conventional cytogenetics, copy number alterations (CNAs) by array comparative genome hybridization, and phenotype changes by cell viability and soft agar assays. Transfection of either the empty vector pcDNA3.1 or pcDNA3.1_*CHI3L1* into 293 cells initiated the punctuated genome changes. In contrast, HeLa_*CHI3L1* cells demonstrated the step-wise genome changes. Increased CIN correlated with lower viability of 293_pcDNA3.1 cells but higher colony formation efficiency (CFE). Artificial *CHI3L1* production in 293_*CHI3L1* cells increased viability and further contributed to CFE. The opposite growth characteristics of 293_*CHI3L1* and HeLa_*CHI3L1* cells were revealed. The effect and function of a (trans)gene can be opposite and versatile in cells with different genetic network, which is defined by genome context. Temozolomide treatment of 293_pcDNA3.1 cells intensified the stochastic punctuated genome changes and CNAs, and significantly reduced viability and CFE. In contrast, temozolomide treatment of HeLa_*CHI3L1* cells promoted the step-wise genome changes, CNAs, and increased viability and CFE, which did not correlate with the ectopic CHI3L1 production. Thus, consistent coevolution of karyotypes and phenotypes was observed. CIN as a driving force of genome evolution significantly influences growth characteristics of tumor cells and should be always taken into consideration during the different experimental manipulations.

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1. Introduction

Chromosome instability (CIN) refers to the rate of genome changes in a given cell population (cell-to-cell variation) and implies clonal chromosome aberrations (CCAs) and non-clonal chromosome aberrations (NCCAs). Generally, CIN correlates with tumorigenic potential of cells, tumor progression, patient survival, treatment sensitivity, and the risk of acquired therapy resistance [1–9]. The studies of the evolutionary process of cancer [4–6,10] revealed that the pattern of genome evolution can be divided into two phases: the Darwinian step-wise continuous phase (marked by step-wise clonal evolution, stable dominant CCAs, and low frequency of NCCAs) and the discontinuous/punctuated phase (marked by elevated NCCAs and transitional CCAs). Shifts between phases are induced by system stress. The high genome-level heterogeneity in the punctuated phase provides the genetic underpinnings of high heterogeneity and clonal diversity universally detected in cancers. NCCAs reflect system instability and drive cancer evolution by increasing population diversity, whereas CCAs actually represent chromosome stability. Furthermore, genome

Abbreviations: aCGH, array comparative genome hybridization; CIN, chromosome instability; CCAs, clonal chromosome aberrations; CNAs, copy number alterations; NCCAs, non-clonal chromosome aberrations; CFE, colony formation efficiency; TMZ, temozolomide.

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heterogeneity in cell population was linked to tumorigenicity [11–13]. Tumorigenicity of cell lines correlated with level of genome heterogeneity regardless of the genetic background of cells. All lines with low tumorigenicity displayed distinctly lower frequency of structural NCCAs [11]. The changing NCCAs/CCAs pattern is a driving force, which ensures genetic heterogeneity, phenotypic plasticity, and population diversity that is essential to cancer evolution. The sequencing studies confirmed that cancer evolution occurs as multiple cycles of the stepwise and punctuated branched polyclonal evolution [14,15].

Depending on the phase, system stresses (the diverse CIN promoting factors) may lead to the very different responses [6]. During the step-wise phase, additional CIN destabilizes the established CCAs and abolishes growth advantage that stemmed from the initial CCAs. Selection and spread throughout the population of the advantageous chromosome aberrations result eventually in fixation of the newly formed dominant CCAs able to continue cancer evolution. During the punctuated phase, additional CIN results in even more increased instability. In such highly stressful conditions most of unstable cells are not viable [6]. Actually, high-level aneuploidy has a negative impact on cellular fitness and generates non-neoplastic and nonviable cells [16,17]. However, chaotic chromosome changes increase the population genome diversity, probability of emergence of the advantageous CCAs, and evolutionary potential of tumor. These phases of cancer cell evolution may shed light on the tumor-promoting and suppressing effects of CIN in tumorigenesis as well as the "paradoxical" relationship between excessive CIN and improved survival outcome in cancer [18,19].

Many cancer-associated genes showed both tumor promoting and suppressing effects (antagonistic functional duality) in the diverse cancer models [20]. The cell type-dependent effects on proliferation, migration, anti-apoptosis, and tumor growth of *CHI3L1* (encoding a secretory glycoprotein chitinase-3-like protein 1, alternatively known as YKL40) were also reported [21–24]. The effect and function of gene can be opposite and versatile in cells with different genomes as gene function is dependent on the genetic network (gene content, RNA and protein expression and their interaction), which is defined by genome context (a number and structure of chromosomes and their nucleus topology) [5,6].

Temozolomide (TMZ) is the cytotoxic alkylating agent for treatment of patients with glioma, melanoma, lymphoma, pancreatic cancer and other types of cancer. TMZ induces methylation of adenine and guanine. If unrepaired, methylguanine mispaires with thymidine, which is recognized by mismatch repair system (MMR). MMR performs errors-prone futile repair cycles resulting in the formation of the secondary lesions, which block DNA replication in the next replication cycle. This leads eventually to DNA double-strand breaks [25,26]. Therapy-mediated stress can significantly change tumor evolution by generating novel phenotypes through induction of genome changes. For example, analysis of chromosomal changes, tumor cell aggressiveness, and chemosensitivity of cell lines established from primary glioma tumors and consecutive recurrences developed under therapy showed genome and phenotype evolution [27]. Similarly, analysis of low-grade gliomas and recurrent TMZ-resistant tumors revealed TMZ-driven hypermutation and evidence of evolution to more aggressive high-grade gliomas [28].

To address these important issues and establish the correlation between changes of karyotype and phenotype, we tracked CCAs/NCCAs pattern, copy number alterations (CNAs), and growth characteristics of 293 and HeLa cell lines after genotoxic stresses (transfection of the empty vector pcDNA3.1, pcDNA3.1_*CHI3L1*, or treatment with TMZ). 293 and HeLa cell lines were reported to be characterized by relatively high karyotype stability during the long-term culture passaging [29,30]. Both cell lines were authenticated (Fig. S1).

2. Materials and methods

2.1. Cell lines

Cell lines were grown in DMEM (HyClone, Thermo Scientific, Logan, UT, USA) supplemented with 10% FBS (HyClone) and 100 U/ml penicillin/100 μg/ml streptomycin (Sigma, USA) in an environment of 95% air/5% CO_2. 293 (HEK293), 293T, and HeLa cell lines were generously gifted by Dr. I. Gout (Department of Structural and Molecular Biology, University College London, London, UK). 293 cells stably transfected with pcDNA3.1 (293_pcDNA3.1 variant 1, 70 passages) and 293 cells stably transfected with *CHI3L1* cDNA in pcDNA3.1 vector (293_*CHI3L1*, 70 passages) were kindly provided by Dr. A. Iershov, clones of HeLa_*CHI3L1* cells by Dr. O. Balynska (Department of Biosynthesis of Nucleic Acids, Institute of Molecular Biology and Genetics, IMBG, Kiev, Ukraine), and 293_pcDNA3.1 (variant 2) cells by Dr. V. Grishkova (Department of Cell Signaling, IMBG). HeLa_*CHI3L1* (clone 1) and (clone 2) were derived from HeLa_*CHI3L1* heterogeneous cell culture on 20th passage of culturing after pcDNA3.1_*CHI3L1* vector transfection (Dr. O. Balynska). 0.8 mg/ml geneticin G418 sulphate (Sigma, St. Louis, MO, USA) was used to select stable cell lines. Antibiotic G418 was withdrawn from cell lines before and during *in vitro* tests. U87 cell line was kindly gifted by Dr. M. Sanson (INSERM, U711, Biologie des Interactions Neurones and Glie, Paris, France). U251 cell line was received from the Bank of Cell Lines from Human and Animal Tissues, R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (Kyiv, Ukraine).

2.2. Long-term treatment with temozolomide (TMZ)

293_pcDNA3.1 (variant 2) and HeLa_*CHI3L1* (clone 2) cells were treated with 100% DMSO-dissolved TMZ (Sigma) during 10 weeks as follows: 10, 20, 40, 60, 80, and 100 μM TMZ for 6 weeks with each concentration two times per week, then 120 μM TMZ two times per week for other 4 weeks, followed by 1 month of washout (TMZ-free medium) before *in vitro* tests. In culture plates DMSO did not exceed 0.4% by volume.

2.3. Conventional cytogenetics

Chromosome samples were prepared by accumulation of metaphases in the presence of 0.5 μg/ml colcemide for 1.5–2 h, followed by treatment with 0.075 M potassium chloride (20 min) and fixation in methanol:acetic acid (3:1, v/v) two times for 30 min each. Fixed nuclei were spread on the wet glass slides (Marienfeld, Germany), air dried and then placed in 5% Giemsa stain (Merck, USA) plus trypsin (HyClone, ThermoScientific, USA) for 4 min. Images of metaphase plates were captured with Olympus BX40 microscope (Japan) and evaluated with Lucia computer analysis system for karyotyping, version 1.6.1 (Laboratory Imaging, Praha, Czech Republic). 100–200 well-spread metaphases were scored per variant to determine chromosome modal number and 20 metaphases were described for chromosome abnormalities. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN 2009).

Modal chromosome number was defined as the most frequent number of chromosomes per cell found across all metaphases studied in a given cell population. CCAs were defined as aberrations found at least in two cells among 20 examined metaphases, whereas NCCAs as aberrations detected in only one cell. Dominant CCAs were ascribed to aberrations with ≥50% clonality (10 or more cells harbored). The frequency of NCCAs in cell line was calculated by using all cells displaying NCCAs divided by the total number of examined cells. Only structural NCCAs were considered.

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