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CLONAL EVOLUTION OF LEUKEMIA CELLS AND CHEMORESISTANCE

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Leukemic cells accumulate mutations and epigenetic changes in the process of disease development forming heterogeneous cell populations that are subject to selection and may respond differentially to therapy. Chemotherapy in turn damages new DNA generating mutations, and may kill leukemia cells most sensitive to drugs and select drug induced resistant subclones. Therefore, it is necessary to monitor changes in the subclonal composition during disease progression as such increased leukemia cell clonal evolution is the main reason for drug resistance and treatment insufficiency. Targeted treatment may be based on the molecular type of disease and on the tested cellular chemosensitivity of the individual patient at least for remission induction. Cellular drug sensitivity evaluation is not alternative to minimal residual disease estimation and whole exome sequencing. As it is not so easy to inhibit tumor evolution, diagnostics of cells sensitivity in the process of evolution may be of particular interest with remission induction. However, advantages of monitoring procedures sometimes are not so obvious.

Key words: *leukemia, clonal evolution, chemoresistance*

High-throughput genomic technologies allow to analyze genomic alterations in tumors on a whole genome scale (DNA copy number changes and nucleotide mutations). Moreover, computational comprehensive method to identify mutator genes and to take into account the increase of the alteration rate by mutator genes, provides more accurate estimates of the tumor age and the timing of driver alterations [1].

Clonal evolution is an intrinsic property of tumor cells that has some traits in common with the conventional accumulation of mutations in body somatic cell populations at aging and at some diseases.

A large number of somatic alterations are detected in tumor genomes, but only some of them are considered as active alterations and drive clonal expansion and invasion. Most of the somatic alterations are neutral for tumor cell selection [2].

Of note, epigenetic alterations in cells are heritable and impact cellular phenotype or physiology that do not occur at the level of alterations in the DNA sequence. These changes effect a set of gene expression and may take part in tumor genesis, heterogeneity and clonal evolution through their specific mechanisms [3]. On the other hand, epigenetic reprogramming may restore cell drug sensitivity.

Leukemic cells accumulate mutations during disease forming heterogeneous cell populations that are subject to selection and may respond differentially to treatment. Therefore, it is necessary to monitor changes in the subclonal composition during disease progression. From this point of view, one should think when the beginning of treatment is more preferable: before or just after the first signs of leukemia progression (it is crucial in CLL but not in acute leukemias). By the way, when the incidence and biological significance of clonal evolution were investigated using conventional and molecular cytogenetics in CLL no correlation was found between clonal evolution and high expression of ZAP70, unmutated IGHV genes or NOTCH1 mutations though clonal evolution and IGHV mutation status had a significant impact on TFS (transformation-free survival). The combination of

conventional and molecular cytogenetics increased the detection of clonal evolution [4].

Clone evolution explains unpredictable leukemia disease course. Leukemia evolution is variable in the types of hematological malignances as well as in patients who have different patterns of leukemia evolution [5].

Leukemia clonal evolution being a fundamental biological process of cell survival through adaptation to the unfavorable microenvironment occurs both during tumor origin and expansion and depends on initial and drug induced intraclonal interaction. It is important to underline that in preleukemia acquisition of leukemogenic mutations occurs in self-renewing hematopoietic stem cells as it was demonstrated by single-cell analysis [6]. It is supposed that most of the mutation found in AML genomes are actually random events that occurred in hematopoietic stem cells before they acquired the initiating mutation. Only one or two additional cooperating mutations are needed to generate the malignant founding clone which in turn can acquire additional cooperating mutations yielding subclones that can contribute to disease progression or relapse [7]. Studies of pediatric acute lymphoblastic leukemia demonstrated that some patients could have multiple genetic subclones of leukemia-initiating cells with a complex clonal architecture [8].

Subclonal diversity at diagnosis provides a variable basis for intraclonal origins of relapse and extended periods of dormancy for stem cells in ETV6-RUNX+ALL [9]. However, not all Ph+ subclones even that persist after hematopoietic stem cell transplantation in Ph+ ALL may have the potential to cause a hematologic relapse [10]. The investigation of clonal heterogeneity in patients with cytogenetically normal acute myeloid with nucleophosmin gene mutation gives the opportunity to reveal that these mutations originate in an early stem cell with both lymphoid and myeloid differentiation potential [11].

Aplastic status which arises in the context of ongoing stem cell damage develops into leukemia through a process of clonal selection and adaptation. Therefore, bone marrow failure may be a risk factor for clonal evolution [12]. The genetic changes that underlie progression from the myelodysplastic syndromes to secondary acute myeloid leukemia studied by the method of whole-genome sequencing indicate that nearly all the bone marrow cells in patients of both groups are clonally derived. Genetic evolution of secondary AML is a dynamic process shaped in multiple cycles of mutation acquisition and clonal selection. Recurrent gene mutations found in both found clones and daughter subclones [13].

Genomic instability includes chromosome instability, increased frequencies of nucleotide mutations and microsatellite instability, which is a special case of this genomic instability and characterized by the expansion or contraction of the number of oligonucleotide repeats present in microsatellite sequences [14].

Chemotherapy damages DNA generating new mutations and may kill leukemia cells most sensitive to drugs and select drug induced resistant subclones. In this connection it is interesting to note that the outcome of CML patients treated with second generation tyrosine kinase inhibitors showed that the hematologic and cytogenetic response rates, 2-year OS and EFS (event-free survival) were not different between patients in chronic phase with and without clonal evolution. However, clonal evolution had a significant adverse impact when associated with other features of accelerated phase [15].

Clonal evolution in relapsed AML revealed by whole genome sequencing brought to light two major clonal evolution patterns: the founding clone in the primary tumor gained mutations and evolved into the relapse or a subclone of the founding clone survived initial therapy, gained additional mutations and expanded at relapse. In all cases, chemotherapy failed to eradicate the founding clone [16]. The other investigation of clonal relationship in AML in various disease phases showed that incomplete eradication of founder clones in the process of treatment rather than stochastic emergence of fully unrelated novel clones underlies relapse and persistence. At the same time cases with two coex-

isting dominant clones of which at least one was chemotherapy sensitive and one resistant were revealed [17].

Therefore, leukemia cell clonal evolution accelerated by treatment should be monitored for cell drug resistance ex vivo before any treatment course by using methods from simple (in suspension culture) to complex (in contact with mesenchymal cells). Moreover, if clonal evolution pattern of each patient is available, its application in clinical practice may show the way to therapy personalization.

Without discussion of the well known drug resistance mechanisms, drug sensitivity monitoring is a method for addition to a new therapy but not instead of it. Most comprehensive analysis in future will be based on the studies of comparative research of normal hematopoietic and leukemic stem cell drug sensitivity in the presence of mesenchymal cells. Currently, most research efforts are put into distinguishing and analyzing driver alterations although an in-depth understanding of the driver alterations in the early stages of tumorigenesis has not emerged for most cancer types. Now we can manipulate the difference of normal and leukemic stem cells drug sensitivity. Therefore, it is reasonable to pay more attention to cells treatment ex vivo in preclinical studies and to compare with the results in vivo.

Targeted treatment may be based on the molecular type of disease and on the tested cellular drug sensitivity of the individual patient at least for remission induction. The amount of drug sensitive cells found in vitro is important for the level of expected treatment response. In the case of therapy relied on the molecular markers, the treatment may involve only a small subclone as there is no reliable information about cell number prepared for response to therapy.

Sometimes there is no coincidence between poor prognostic molecular markers and good survival [5, 18]. It should be stated that ex vivo cell drug sensitivity studies have their shortcomings [19-22]. Therefore, multilevel drug sensitivity diagnostics are to be investigated.

It seems that is no need to be in search of new separate prognostic molecular markers. It is necessary to foresee the first and next cell responses to therapy on the basis of cell susceptibility studies namely at the moment of treatment beginning and in its course in order to reach remission. This approach gives the opportunity to take into consideration the integral response without costly and time consuming comprehensive study of mutational and epigenetic evolution mechanisms and has clinical application despite all disadvantages of the methods used.

Leukemia cases usually don't share absolutely all the same genomic features. The personalized therapy connected with the unstable individual profile of cellular drug susceptibility is of definite sense. Minimal residual disease estimation and whole exome sequencing are not alternative to cellular drug sensibility evaluation.

As it is not so easy to inhibit tumor evolution, diagnostics of cells sensitivity in the process of evolution may be of particular interest with remission induction. However, advantages of monitoring procedure sometimes are not so obvious.

From a practical standpoint it should be stated that clinical efficacy depends on whole-genome sequencing or at least on genome-wide associated studies, minimal residual disease evaluation and direct testing of cell drug sensitivity at the appropriate periods of the disease.

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КЛОНАЛЬНАЯ ЭВОЛЮЦИЯ ЛЕЙКОЗНЫХ КЛЕТОК И ХИМИОРЕЗИСТЕНТНОСТЬ

Лейкозные клетки накапливают мутации и эпигенетические изменения в процессе развития заболевания, формируя гетерогенные популяции клеток, которые являются объектом выбора для воздействия и могут по-разному реагировать на терапию. Химиотера-

пия, в свою очередь, воздействует на вновь образованные мутации ДНК и может уничтожать наиболее чувствительные к лекарственным препаратам лейкозные клетки, сохраняя химиоиндуцированные устойчивые субклоны. Поэтому необходимо следить за изменениями в субклональной структуре во время прогрессирования заболевания, так как нарастающая клональная эволюция лейкозных клеток является основной причиной лекарственной устойчивости и неэффективности лечения. Таргетная терапия базируется на учете молекулярного типа заболевания, тогда как цитотоксическая терапия – на определяемой клеточной химиочувствительности пациентов. Оценка клеточной чувствительности к лекарственным препаратам не является альтернативой определению минимальной остаточной болезни или полного секвенирования генома для выбора терапии. Так как нелегко ингибировать эволюцию генома опухоли, определение чувствительности клеток в процессе опухолевого роста может оказаться важным при индукции ремиссии. Тем не менее, преимущества отдельных процедур мониторинга не всегда очевидны.

Ключевые слова: *лейкоз, клональная эволюция, химиорезистентность*

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