Mitotic crossover promotes leukemogenesis in children born with TEL-AML1 via the generation of loss of heterozygosity at 12p

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Abstract

TEL-AML1 (ETV6-RUNX1) fusion gene which is formed prenatally in 1% of the newborns, is a common genetic abnormality in childhood B-cell precursor acute lymphoblastic leukemia. But only one child out of a hundred children born with this fusion gene develops leukemia (bottleneck phenomenon) later in its life, if contracts the second mutation. In other words, out of a hundred children born with TEL-AML1 only one child is at risk for leukemia development, which means that TEL-AML1 fusion gene is not sufficient for overt leukemia. There is a stringent requirement for a second genetic abnormality for leukemia development and this is the real or the ultimate cause of the leukemia *bottleneck* phenomenon. In most cases of TEL-AML1+ leukemia, the translocation t(12;21) is complemented with the loss of the normal TEL gene, not involved in the translocation, on the contralateral 12p. The loss of the normal TEL gene, *i.e.* loss of heterozygosity at 12p, occurs postnatally during the mitotic proliferation of TEL-AML1+ cell in the mitotic crossing over process. Mitotic crossing over is a very rare event with a frequency rate of 10^{-6} in a 10 kb region. The exploration and identification of the environmental exposure(s) that cause(s) proliferation of the TEL-AML1+ cell in which approximately 10^6 mitoses are generated to cause 12p loss of heterozygosity, *i.e.* TEL gene deletion, may contribute to the introduction of preventive measures for leukemia.

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Introduction

Curing a cancer such as acute lymphoblastic leukemia can incur a price for a young patient in physical or intellectual development. And even if this was not the case, leukemia and cancer research driven by the allure of miracle cures is impoverished indeed if it does not pay equal attention to possible causal mechanisms and prospects for prevention. Childhood leukemia is a clinical success story, with greater than 80% cure rate, depending on the phenotype and tumor genetics. However, most cured children face long term sequelae such as heart defects or chronic ailments[1] and prevention of the disease is our ultimate goal. The idea of leukemia prevention is not new. The idea was launched by Alfred Knudson almost fifty years ago.[2] The current knowledge concerning natural history of childhood leukemias has substantially changed our understanding of the disease and more importantly has brought the prospect of its prevention. This is especially true for TEL-AML1+ childhood acute lymphoblastic leukemia (ALL), which in many aspects is the most and the best studied type of childhood leukemia. With this paper we want to offer some further, new light in the most mysterious phenomenon of childhood leukemia, phenomenon of leukemia *bottleneck*.

TEL-AML1+ childhood acute lymphoblastic leukemia: two hit disease

TEL-AML1 fusion gene generated via the t(12;21)(p13:q21) chromosomal translocation – which is a common genetic abnormality in childhood B-cell precursor ALL present in 20-25% of cases[3,4] – occurs prenatally.[5] Without this fusion gene there is no TEL-AML1+ ALL, but alone, this fusion gene is not sufficient for overt leukemia.[6] In 2002 it was disclosed that 1% of the healthy newborns carry TEL-AML1 fusion gene, but only 1% of these newborns later in their life develops leukemia.[5] This phenomenon was called *bottleneck* of childhood

leukemia. In our recently published paper we have presented our mathematically postulated explanation for this phenomenon.[7] According to our postulation, childhood leukemia bottleneck is caused by a very low total body TEL-AML1+ cell count, which is a corollary of the very late fetal TEL-AML1 fusion gene generation. To the best of our knowledge, this is the only explanation for this phenomenon, published so far. To be more clear, in our paper we have just explained why only one child out of a hundred children born with TEL-AML1 is at risk for leukemia development later in its life. Whether this child will develop leukemia or not depends exclusively on whether this child will contract the second mutation or not. That being said, the real bottleneck is caused by stringent requirement for additional, second, genetic abnormality to complement the first, prenatally acquired t(12;21)(p13:q21) chromosome translocation. Most patients with TEL-AML1+ ALL at diagnosis have loss or deletion of the normal copy of TEL gene-allele on the contralateral 12p (del(12p)), not involved in the translocation.[6] Del(12p) occurs postnatally in more mature cells[8] under exposure of an environmental factor so far unknown. In the absence of contralateral TEL gene, TEL-AML1+ cell becomes leukemic owing to the unrestrained and uncontrolled TEL-AML1 protein activity.[9] This second mutation seems to be rate-limiting and is the ultimate cause of the bottleneck phenomenon of childhood TEL-AML1+ ALL.[6]

Loss of heterozygosity of the TEL-AML1+ cell

At birth a child born with TEL-AML1 fusion gene, from a genetically point of view, has a single naïve TEL-AML1+ cell is in the state of heterozygosity at 12p for TEL gene. When this cell experiences the second mutation, *i.e.* the loss of the normal, noninvolved in the translocation of TEL gene, which is inevitable for leukemogenesis in most children developing TEL-AML1+ ALL,[6] the cell enters into the state of so called, *the loss of heterozygosity* (LOH) at 12p. Loss of heterozygosity is a hallmark of numerous cancers,[10-14] and it refers to change from a state of heterozygosity in a normal genome to a homozygous state in a paired genome. Loss of heterozygosity is most often regarded as a mechanism for disabling tumor suppressor genes (TSGs) during the course of oncogenesis.[15,16] That being said, LOH results in no normal tumor suppressor being produced that often results in tumorigenesis.[17] Deletion of the normal TEL gene must have some potent selective advantage in cells carrying TEL-AML1 fusion, which might relate either to a suppressor function of TEL gene[18] or to the ability to dimerize with TEL-AML1 and reduce its transforming activity.[19] As a consequence, in the absence of the contralateral TEL gene function, TEL-AML1+ cell becomes leukemic, owing to the unrestrained and uncontrolled TEL-AML1 fusion protein activity.[9]

Mitotic cross over causes loss of heterozygosity at 12p

After a comprehensive prospective screening of leading journals for leukemia and cancer that we did, it could be concluded that mitotic cross over (MCO) could be one of the crucial mechanisms that cause 12p LOH in naïve TEL-AML1+ cell. Generally, it has been proved that many cases of LOH are caused by mitotic recombination (MR) between homologous chromosomes.[20] Historically it was a surprise for geneticists to discover that crossing-over can also occur at mitosis. Presumably it must take place when homologous chromosomal segments are accidentally paired in asexual cells such as body cells (including naïve B cells and TEL-AML1+ B cells). Mitotic cross over is a rare, but it is important in some organisms – for example, some fungi that do not have a sexual cycle use mitotic crossing-over as a source of variation. Mitotic cross over occurs only in diploid cells such as the body cells of diploid organisms and it is a very rare event because it happens at frequency rate of ~10^{-6} in a 10 kb locus between repeated DNA sequences in each cell per cell cycle.[21] In other words, there is a need for ~10^6 mitoses to ensue only one MCO in a 10 kb locus. In TEL-AML1+ ALL, TEL deletions vary in size from 10 kb to>10 megabases. Since minimally deleted regions always affect at least some part of the TEL transcriptional framework,[22] it could be concluded that reported frequency of ~10^{-6} MCO in a 10 kb locus are valid for MCO frequency at 12p in naïve TEL-AML1+ cells, as well. Finally, the presented *mitotic cross over* hypothesis of generating second leukemogenic mutation is directly supported by the fact that no tumour-cancer has been seen so far from the cells that underwent terminal differentiation, *i.e.* the cells that have no more mitotic capability. The cells having this property are for example, alpha motor neurones of the anterior horns of medulla spinalis, Purkinje cells of the cerebellar cortex, and motor neurons in the motor region of the cerebral cortex (Betz neurons) or in the brain stem. These cells do not have capability for further mitotic activity and therefore: i) are not exposed to MCO events; ii) cannot contract second-hit mutation, *i.e.* LOH; and iii) consequently cannot deliver cancer. It is very well known that persons having Li-Fraumeni syndrome are prone to various cancers owing to the heterozygous germline p53 mutation, that is distributed in all cells, including previously mentioned cells. Having this in mind, we did very extensive search of medical literature on the tumors originating from the cells that underwent terminal differentiation, but we found no tumor from these cells. To the best of our knowledge we are the first and so far the only one, with written statement in the world medical literature, to explain why there is no cancer in cells that are deprived from further mitotic activity.[23]

The corollary of the postulation

Provided that the presented mathematical postulation and our previous one[7] are correct, one very important corollary must be harbored. And that is the existence of substantial number of congenital TEL-AML+ ALL (practically all children born with TEL-AML1 fusion gene should be born with overt leukemia). We have previously shown that each child born with TEL-AML1 fusion gene has approximately 10^8 TEL-AML+ body B cells.[7] Having in mind the MCO frequency of ~10^{-6} and the fact that 10^8 TEL-AML+ body B cells are generated through 26 mitotic steps including approximately 10^8 mitoses, it follows that 100 TEL-AML1+ B cells should have contracted 12pLOH(10^8/10^6) and subsequently become leukemic. But no case of congenital TEL-AML+ ALL has been reported so far. The only reasonable explanation for this could be the assumption that during the fetal lymphopoieseis in the *sterile* intrauterine cavity, an unknown biological mechanism protects proliferating TEL-AML1+ B cells from the MCO event at 12p and from contracting 12pLOH, *i.e.* deletion of the normal TEL gene not involved in the t(12;21)(p13:q21) chromosomal translocation.

Conclusions

Leukaemia bottleneck is caused by two independent events. The first event is generated prenatally during the fetal development, when the first event has been shown to occur. The second one is the stringent need for the second mutation, *i.e.* the deletion of the normal TEL gene occurring postanatally during the process of mitotic crossing over in B cells carrying TEL-AML1. The exploration and identification of the post-

natal environmental exposure(s) that cause(s) the proliferation of TEL-AML1+ cell – in which approximately 10^6 mitoses ensue, which enables the generation of the second mutation, *i.e.* 12pLOH, and consequently the loss of the contralateral, normal TEL gene not involved in the translocation – may contribute to the introduction of leukaemia preventive measures.

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