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Screening for gene mutations: will identification of *NT5C2* mutations help predict the chance of relapse in acute lymphoblastic leukemia?

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“...the discovery of *NT5C2* mutations in both B- and T-lymphoblastic leukemia demonstrates the power of selective pressure of chemotherapy in spite of dramatically different biological subtypes.”

Great strides have been made over the past several decades in improving the outcome of childhood acute lymphoblastic leukemia (ALL) making it one of the most curable childhood cancers. Overall survival rates now approach 90% [1]. Despite these triumphs, disease recurrence still presents a major challenge as 10–20% of patients fail frontline therapy. Such patients have a dismal prognosis even with aggressive retrieval strategies including stem cell transplantation making relapsed ALL a common cause of cancer death in children [2, 3]. Thus, novel approaches to prevention and treatment are urgently needed.

Advancements in genome technology have made it possible to study the evolution of ALL from diagnosis to relapse. Such studies have led to the discovery of key components or

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pathways that are associated with chemotherapy resistance [4–6]. Adding to this growing field of research, we and others recently discovered relapse-specific mutations in *NT5C2*, a gene that directly influences chemosensitivity [7, 8].

We performed whole transcriptome sequencing, followed by full exon sequencing, on matched diagnosis and relapse bone marrow specimens of pediatric B-lymphoblastic leukemia (B-ALL) patients to identify relapse-specific mutations in *NT5C2* in approximately 10% of individuals [7]. Similarly, in another recent study, mutations in *NT5C2* were the most common somatic alteration in 19% of relapsed T-lymphoblastic leukemia (T-ALL) and 3% of relapsed B-ALL patients [8].

NT5C2 is a 5′-nucleotidase enzyme that is active in the cytoplasm of cells and is responsible for the maintenance of intracellular nucleotide pools. In total, seven proteins belong to this class of enzymes, each with a specific location and function. As an intracellular nucleotidase, *NT5C2* shows substrate specificity for guanine monophosphate and inosine monophosphate-based nucleotides as well as deoxyguanine and deoxyinosine monophosphate forms, catalyzing the dephosphorylation of these substrates to nucleosides that can then be shuttled out of the cell [9, 10].

Remarkably, all patients carrying *NT5C2* mutations relapsed early (within 36 months from diagnosis), a subgroup of patients who fare extremely poorly with current salvage regimens. These mutations appear to be gain-of-function mutations leading to increased enzymatic activity of 5′ nucleotidase, resulting in the dephosphorylation and inactivation of cytotoxic metabolites of purine nucleoside analogues 6-mercaptopurine and 6-thioguanine, a major component of current maintenance therapy. To determine the origin of these mutations, we backtracked mutations in B-ALL patients who harbored relapse-specific mutations using ultra deep sequencing and identified two out of seven cases, where a rare clone existed at diagnosis [7]. With these results and the fact that these patients relapse early in treatment, we speculate that clones containing *NT5C2* mutations exist at diagnosis in all cases and emerge when 6-mercaptopurine assumes a major role in treatment (e.g., maintenance).

“...all patients carrying *NT5C2* mutations relapsed early (within 36 months from diagnosis).”

These findings highlight the clonal heterogeneity of ALL. While risk stratification based on cytogenetic and molecular genetic features of the dominant clone have led to important strides in improving the overall outcome, minor subclones may represent an impending threat. In addition, the discovery of *NT5C2* mutations in both B- and T-ALL demonstrates the power of selective pressure of chemotherapy in spite of dramatically different biological subtypes. This scenario is reminiscent of convergent evolution in different species in the natural world.

How might these findings lead to new approaches to the prevention and treatment of relapse? First, inhibitors of wild-type *NT5C2* have been identified that contain structures similar to the phosphate groups that the enzyme hydrolyzes [11]. The lead inhibitor to date, anthraquinone-2,6-disulfonic acid, shows strong inhibition of *NT5C2* in the high micromolar range in cell lines. The specificity of the compound for *NT5C2* *in silico* is also

strong but it remains to be determined how this compound affects other 5' nucleotidases in the cell. In addition, it may be possible that other inhibitors may be developed that more specifically target the mutant enzyme as the effect of anthraquinone-2,6-disulfonic acid on mutant NT5C2 remains unknown.

Second, *NT5C2* mutations confer resistance to a class of agents that are the backbone of maintenance therapy and perhaps early detection of such mutations could be used as a biomarker of impending relapse. Based on this information, clinicians could alter treatment to emphasize noncross resistant therapy. To further address this question, *in vivo* xenograft modeling will be useful to determine if treatment with purine analogs results in the outgrowth of a minor clonal population present at the time of engraftment and further, whether relapse can be aborted with a switch to noncross resistant chemotherapy agents before the clinical detection of relapse. Potentially cost-effective and sensitive methodologies are emerging to allow early identification of emerging mutations while the patient is in morphological remission. Recent advances in genomic technologies have progressed at a rapid pace, accompanied by plunging costs, allowing investigators to address wide ranges of biologically important questions.

“...detection of such mutations could be used as a biomarker of impending relapse.”

A major caveat, however, is that additional data from many laboratories including ours indicate that relapsed blasts acquire resistance to multiple drugs. Thus, the identification of additional drivers of chemoresistance will be needed to develop a comprehensive approach to the screening and prevention of drug resistance in ALL.

In a nutshell, modern high-throughput genomic techniques are providing valuable insight into the clonal evolution of cancer and the discovery of driver mutations responsible for relapse will lead to novel therapeutic strategies.

Biographies

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