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Detoxification enzyme polymorphisms as risk factors fo t-AML

MARIA TERESA VOSO
FRANCESCO D'ALO'
GIUSEPPE LEONE

Istituto di Ematologia, Università Cattolica del Sacro Cuore, Roma, Italy

To date, therapy-related myelodysplastic syndromes/acute Myeloid Leukemias (t-MDS/AML) represent the main long-term adverse effect of cancer treatment. The identification of new prognostic factors has raised the question whether reducing treatment intensity in good prognosis patients may save them from long term-toxicity, without impairing response rates.

Although the genotoxic effects of ionizing radiation and chemotherapic drugs play the central role in pathogenesis of t-MDS/AML, individual susceptibility factors can help to identify patients at increased risk. In particular, genetic polymorphisms in genes codifying for drug metabolizing and DNA repair enzymes may interfere with correct working of physiological processes that protect cells against drug- and radiation-induced genomic damage and may contribute to explain why some patients develop secondary neoplasias and others do not.

Drug or xenobiotic metabolizing enzymes (DME) include several enzymatic families involved in the metabolism, biotransformation, and detoxification of xenobiotics and foreign compounds. They display a high degree of polymorphism in the general population. Two main DMA classes have been identified, named Phase I and Phase II enzymes, respectively. Phase I DMEs primarily consist of the cytochrome P450 (CYP) superfamily, which is found in abundance in the liver, gastrointestinal tract, lung and kidneys, and consists of families and subfamilies classified based on their amino acid sequence identities or similarities. These enzymes participate to first steps of detoxification from xenobiotic and are frequently responsible of their activation to reactive intermediates that can form adducts with DNA, leading to its damage.[1]

The Phase II drug metabolizing or conjugating enzymes consist of many superfamily of enzymes, including sulfotransferases, glucuronosyltransferases, NAD(P)H:quinone oxidoreductase (NQO), epoxide hydrolases (EPH), glutathione S-transferases (GST) and N-acetyltransferases (NAT). Generally, Phase II affect detoxification and ultimately excretion and elimination of many drugs and xenobiotics that contain hydroxyl (OH) functional groups, highly reactive, either present on the parent molecules and/or after biotransformation by the Phase I enzymes. For instance, reactive electrophiles are typically conjugated with glutathione (GSH) by various GSTs, and when GSH levels in the cells are attenuated, DNA and other cellular constituents are at high risk of damage.[1]

Several Phase I and Phase II enzymes are involved in detoxification of chemotherapic agents and their polymorphisms have been often correlated to modified risk of t-MDS/AML. Epipodophyllotoxins, as well as cyclophosphamide, ifosphamide, vinblastine, and vindesine are substrates for metabolism by CYP3A, the most abundant component of the CYP system in the human liver.[2] A variant in the 5' promoter region of the CYP3A4 gene (CYP3A4-V), associated to reduced intracellular enzyme level, decrease production of the epipodophyllotoxin catechol metabolite, which is the precursor of the potentially DNA-damaging quinone. Accordingly, Felix et al showed that 19% *de novo* and only 3% treatment-related leukemias carried the CYP3A4-V, and that the variant was absent in patients with MLL gene translocation.3 These data were confirmed by Rund *et al.* Individuals carrying the CYP3A4-*wild type* genotype may have increased production of potentially DNA-damaging reactive intermediates and are at

increased risk for t-AML.[4]

In consideration of well known toxic effects of polycyclic aromathic hydrocarbons on blood and bone marrow, benzene represents an excellent model for secondary carcinogenesis. Benzene is metabolized by the phase I hepatic enzyme CYP2E1 to benzene oxide, which is itself further metabolized to hydroquinone. Hydroquinone and related hydroxy metabolites are are potent hematotoxins and genotoxins that can be converted by the enzyme NAD(P)H:quinone oxidoreductase (NQO1) to less toxic hydroxylmetabolites. Looking at toxicity due to benzene exposure in benzene workers over a 16-month period, Lan *et al.*, found that total blood counts and colony formation from myeloid progenitor cells significantly declined with increasing benzene exposure. Hematologic impairment was significantly associated to single-nucleotide polymorphisms in the MPO –463GG and NQO1 465CT genes, in particular when combined.[5] Accordingly, Larson et al showed that the frequency of NQO1 heterozygotes was higher among leukemia patients than expected in the general population, and homozygotes mutants were 4% in patients with primary AML, and 11% in t-AML.[6] A model for carcinogenesis due to NQO1 defect is that of infant t-ALL, which is supposed to arise *in utero* and to be influenced by maternal exposure to carcinogens. In these children, a higher frequency of MLL translocations and of NQO1 C609T polymorphism was reported.[7]

Many cytostatic drugs such as adriamycin, BCNU, bleomycin, chlorambucil and cyclophosphamide are inactivated by GST. Several GST are polymorphically expressed, in particular the GSTP1 gene present a variant allele, with a substitution of isoleucine to valine at amino acid codon 105 (Ile105Val), which occurs at a frequency of about 30% in Caucasian populations and is associated with a decreased activity of the enzyme. In central Europe GSTM1 is homozygously deleted in about 50% and GSTT1 in 20% of Caucasian individuals. Individuals with a GSTM1 and/or GSTT1 or NAT polymorphisms show a greater level of DNA damage following carcinogen exposure, as determined by sister chromatid exchange (SCE) and formation of DNA-adducts.[8] Sasai et al reported an increased risk for t-AML for Japanese patients with the GSTT1 null genotype. In a retrospective study, 213 patients with AML and 128 with MDS, 44 of whom therapy-related, were compared to 239 healthy individuals.[9] A significant over-representation of combined deletions of GSTM1 and GSTT1 was found in t-AML/MDS secondary to chemo- and/or radiotherapy for breast cancer.[10] In our patient series, we did not find any difference in the prevalence of GSTM1 and/or GSTT1 deletions between *de novo* and t-AML patients, suggesting that both other factors and the racial context may interfere on the risk associated to these genotypes.[11] In the same lane Allan et al, examining 89 cases of t-AML, 420 cases of *de novo* AML, and 1022 controls, found that GSTM1 or GSTT1 deletions were not specifically associated with susceptibility to t-AML, while individuals with at least one GSTP1 codon 105 Val allele were significantly over-represented in t-AML cases. In particular, the highest t-AML risk was present in patients with GSTP1 codon 105Val allele with prior exposure to chemotherapy, particularly to known GSTP1 substrates, and not among those t-AML patients with prior exposure to radiotherapy alone.[12]

Although DMEs can protect DNA from interaction with highly reactive and potentially harmful xenobiotics and drugs, cells are further protected from genotoxic stress by DNA repair pathways. The fine regulation of these processes is of outstanding interest because their alterations are recognized as important mechanism of secondary leukemogenesis. In particular, double-strand breaks (DSBs) in DNA are the most important class of DNA damage because they may lead to either cell death or loss of genetic material, resulting in chromosomal aberrations. Too little repair leads to the acquisition and persistence of mutations, whereas elevated levels of repair can inhibit the apoptotic pathway and enable a cell with damaged DNA to attempt repair, potentially mis-repair, and survive. One of the central proteins in the DNA-repair pathway is RAD51, which binds to DNA and promotes ATP-dependent homologous pairing and strand transfer reactions. The RAD51 gene presents a G/C polymorphism (RAD51-G135C) which is associated to increased enzyme level and can potentially alter the stechiometry of DNA reapir reaction. The XRCC3 protein also functions in DSB repair pathway and directly interacts with and stabilizes RAD51. A polymorphism at codon 241 in the XRCC3 gene results in a Thr-to-Met amino acid substitution and this variant allele has been associated with higher level of DNA adducts, compared with the wild-type gene. In 51 t-AML patients, the frequency of the RAD51-G135C polymorphism was significantly higher than in controls, translating in a 2.66-fold increased t-AML risk. This risk was even higher when the RAD51 polymorphism was combined with at least

one variant XRCC3-241Met allele. Moreover, a combined detoxification/DNA repair defect is probably to further modify the risk of t-AML, as observed for the combination of GSTM1-deleted genotype with the double RAD51-G135C/XRCC3-241Met, which was associated to a 15-fold increased risk of t-AML development.[13] These results lead to the tempting hypothesis that reactive species escaping detoxification mechanisms or produced in excess due to drug metabolizing enzymes polymorphisms, damage DNA which is inefficiently repaired due to defective DNA-repair.

In conclusion, the large amounts of studies produced in last years have identified polymorphic variants of DMEs and DNA repair enzymes able to modify the individual susceptibility to develop t-MDS/AML after chemo- and/or radiotherapy. However, since different studies have so far produced contradictory results due to heterogeneity of populations, wider studies need to clarify the impact of single and combined enzymatic polymorphisms on t-MDS/AML risk. In future, one of the most interesting challenge in cancer therapy will be to tailor treatment to patients' genetic background.

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