Genetics of high-dose methotrexate-induced oral mucositis: current perspectives

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High-dose methotrexate (HD-MTX) is part of routine care to patients with childhood and adult cancers such as lymphoma and acute-lymphoblastic leukemia. MTX is a folate-antagonist and inhibits the folate pathway leading to impaired DNA synthesis and cell death. Overall survival rate has increased dramatically since the introduction of HD-MTX to standard care. However, a large number of patients suffer from toxicity such as oral mucositis, which occurs in about 20% of the patients treated with HD-MTX [1]. Folinic acid (a folate vitamer) is prescribed to reduce toxic side effects of HD-MTX therapy. Identification of genetic variants that can predict occurrence of mucositis would aid in lowering toxicity and increased survival of patients treated with HD-MTX.

Methotrexate uptake & metabolism

MTX is taken up by the reduced folate carrier (SLC19A1) and PCFT and is polyglutamated intracellularly by FPGS leading to up to seven polyglutamated chains (MTX-PG1-7) [2]. This polyglutamation of MTX renders MTX in the cell, which improves efficacy but also increases toxicity, as higher intracellular MTX levels can be reached [2]. MTX is most efficiently exported as MTX short chain polyglutamates (MTX PG1-3), and therefore, long-chain polyglutamates (MTX PG4-7) first need to be cleaved by GGH before they can be exported by ABC-transporters [2]. Intracellularly, MTX inhibits DHFR and TYMS; thereby, impairing purine- and pyrimidine synthesis, which leads to apoptosis of tumor cells. Folinic acid is a folate derivative which bypasses the DHFR block by MTX, and thereby, decreases toxicity rates of healthy cells. As MTX inhibits DHFR, this also has implications for one-carbon metabolism as less one-carbon groups are available for remethylation of homocysteine into methionine, and subsequently, availability of methyl groups for cellular methylation reactions [3].

Pharmacogenetics of high-dose methotrexate in relation to oral mucositis

Most candidate studies have focused at genes involved in pharmacokinetics (PK) and pharmacodynamics (PD) of HD-MTX in relation to mucositis. One of the most studied variants is the c.677 C>T variant in the gene coding for *MTHFR* (rs1801133). *MTHFR* 677 TT genotype has been associated with lower circulating folate levels, and therefore, is a good candidate gene to study in regard to MTX toxicity [4]. Several studies investigated the *MTHFR* c.677C>T variant in relation to oral mucositis but results are inconsistent. A recent meta-analysis demonstrated a significant association of the *MTHFR* 677 T allele with oral mucositis in children with malignancy [5].

Besides *MTHFR* also variants in *TYMS* have been frequently studied. We recently studied two common SNPs in the *TYMS* gene (rs2853542, 28 bp repeat 3RG>C and rs151264360, 6 bp del TTAAAG), which are involved in pyrimidine synthesis. A meta-analysis of four studies showed that the *TYMS* 2R3R and 3R3R genotypes were not significantly associated with acute lymphoblastic leukemia (OR: 1.45 [0.99–2.14]) [6]. In addition, SNPs in miRNA coding genes have been studied and the miR-1206 genetic variant (rs2114358) has been associated with oral mucositis in acute lymphoblastic leukemia patients [7,8]. Several other variants in PK/PD have been studied

like genetic variation in *SLC19A1*, also known as reduced folate carrier (*SLC19A1/RFC*) and *FPGS* in relation to MTX plasma levels or oral mucositis, but most studies need replication to confirm their role in MTX-induced oral mucositis toxicity [9,10].

In addition to genetic variants, we recently assessed changes in DNA methylation in relation to MTX-induced oral mucositis. DNA methylation is dependent upon the availability of methyl groups, which are donated through folate-dependent one-carbon metabolism. MTX not only inhibits purine- and pyrimidine synthesis but also inhibits one-carbon metabolism [11,12]. As one-carbon metabolism donates methyl groups for all cellular methylation reactions, it can be hypothesized that MTX leads to impaired DNA methylation. Global DNA methylation was assessed by *LINE-1* elements, which are a proxy for global DNA methylation, and in addition, two important metabolites of one-carbon metabolism, which are involved in the regulation of cellular methylation (S-adenosylmethionine [SAM] and S-adenosylhomocysteine [SAH]), were measured in acute lymphoblastic leukemia (ALL) patients before and after MTX therapy. Interestingly, plasma SAM levels increased and *LINE-1* methylation decreased during MTX therapy but this was not associated with MTX-induced oral mucositis in ALL patients [3]. Future epigenome-wide association studies are of interest to assess the involvement of DNA methylation in MTX-induced oral mucositis.

Challenges in pharmacogenetics of methotrexate-induced oral mucositis

Pharmacogenetics has been shown useful in predicting toxicity of drugs influencing pyrimidine and purine synthesis, like 5-fluorouracil, by genetic variants in the gene coding for *DPYD* and azathioprine by genetic variants in *TPMT* [13,14]. However, MTX-induced toxicity is a complex interaction between disease and drug response, in which folate-rescue therapy is also given, which is relevant in predicting MTX toxicity. Most studies focused on pharmacogenetics of MTX-induced oral mucositis as an end point. We and others have developed methods to measure MTX and its polyglutamates (MTX-PG) intracellularly in erythrocytes using tandem mass spectrometry, which is of potential interest to study in relation to oral mucositis [15]. One recent genome-wide association study investigated MTX clearance from plasma and identified *SLCO1B1* in children with ALL [16,17], which seems promising in MTX-induced oral mucositis [18]. However, genetic association studies of MTX toxicity are hampered by low sample size, which enables discovery of genetic variants, but often lacks replication of genetic loci. In future, functional studies are warranted that assess the role of genetic variants in relation to intermediate phenotypes such as (intracellular) MTX levels and clinical end points such as oral mucositis. In addition, next-generation sequencing of PK/PD genes is a valuable approach to identify rare genetic variants that could predict MTX toxicity in a more personalized way.

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