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Role of microRNAs in fluoropyrimidine-related toxicity: what we know

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Abstract. – Although more than half a century has passed since the discovery of fluoropyrimidines, they are still used in the treatment of many types of cancer, and it is estimated that annually two million patients undergo fluoropyrimidine-based chemotherapy. The toxicity resulting from the use of fluoropyrimidines affects about 30-40% of patients, which in some cases may prove to be lethal. The key player in fluoropyrimidine toxicity is DPD activity, and patients with deficits are more likely to develop significant adverse events. In addition to genotyping DPYD variants associated with DPD deficiency, overexpression of miR-27 has also been shown to be a predictive factor for fluoropyrimidine toxicity. This review aims to relate what we know so far about the involvement of miRNA in fluoropyrimidine toxicity and to open new perspectives in this field.

Key Words:
Fluoropyrimidine, Chemotherapy, Toxicity, Pharmacogenetics.

Introduction

Fluoropyrimidines are antimetabolite drugs widely used in the treatment of head and neck cancer, breast cancer, esophageal cancer, gastric cancer, pancreatic cancer, hepatobiliary cancer, colorectal cancer, anal cancer, and vulvar cancer[1]. The fluoropyrimidines used in clinical practice are 5-fluorouracil (5-FU), Capecitabine, Tegafur, S-1 (tegafur/gimeracil/oteracil), and TAS-102 (trifluridine/tipiracil). 5-FU and its oral prodrug, Capecitabine, are the most commonly used fluoropyrimidines, Tegafur and S-1 are mostly used in Asia, and TAS-102 is used in subsequent treatment lines upon the failure of classical fluoropyrimidines and targeted therapy.

The prodrugs Capecitabine, TAS-102, S-1, and Tegafur are converted to 5-FU, which is subsequently activated in several steps to FdUMP (fluorodeoxyuridine monophosphate), which is an inhibitor of TS (thymidylate synthase)[1,2]. TS inhibition leads to the depletion of dTMP (deoxythymidine monophosphate) but an accumulation of dUMP (deoxyuridine monophosphate). FdUMP and dUMP can be phosphorylated to FdUTP (fluorodeoxyuridine triphosphate) and dUTP (deoxyuridine triphosphate), respectively, and incorporated into DNA[1,2]. TAS-102 is the combination of TFT (trifluridine) and TPI (tipiracil hydrochloride). TFT can be phosphorylated to TF-thymine, which can be inhibited by TPI. TFT is phosphorylated to TFT-MP (trifluridine-monophosphate), which is a reversible inhibitor of TS, while upon phosphorylation to TFT-TP (trifluridine-triphosphate), it can also be incorporated into DNA. Substitution of dTTP (deoxythymidine triphosphate) by either TFT-TP or dUTP or FdUTP leads to DNA damage and cell death[3].

Capecitabine and Tegafur are converted to 5-FU by thymidine-phosphorylase (TP) and CYP2A6. 1-5% of 5-FU is transformed into cytotoxic metabolites FdUMP, FdUDP (fluorodeoxyuridine diphosphate), FdUTP and FUTP (fluorouridine triphosphate)[1,2]. 5-FU is directly transformed to FUMP (fluoridine monophosphate) by OPRT (orotate phosphoribosyltransferase) or indirectly through FUR (fluorouridine) by UP (uridine phosphorylase) and UK (uridine kinase). FUMP is then phosphorylated

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to FUDP (fluorouridine diphosphate), which is phosphorylated to FUTP. FUDP can also be converted to FdUDP by ribonucleotide reductase (RR)[1,2]. In an alternative activation pathway, 5-FU is converted to FUDR (fluorodeoxyuridine) by thymidine phosphorylase. FUDR is converted through thymidine phosphate in FdUMP, which is phosphorylated to FdUDP, which is phosphorylated to FdUTP. Eighty percent of the 5-FU is catabolized by dihydropyrimidine dehydrogenase into the non-cytotoxic metabolites DHFU (5-fluoro-5,6-dihydrouracil), which is then converted to FUPA (fluoro-beta-ureidopropionate) and FUBA (fluoro-beta-alanine), which are eliminated through urine. To modulate the activity of fluoropyrimidines, inhibitors of DPD can be administrated, which slow the degradation of 5-FU and improve the response rate[1,2] (Figure 1).

The rate-limiting enzyme of 5-FU catabolism is dihydropyrimidine dehydrogenase (DPD), which metabolizes almost 80% of the dose of 5-FU and Capecitabine[4]. DPD is the essential enzyme for the 5-fluorouracil catabolism, and therefore the amount of 5-FU converted in cytotoxic metabolites depends on the systemic activity of DPD[4]. Some of the severe side effects that occur during fluoropyrimidine treatment are due to metabolism through the DPD. Deleterious genetic variants in the gene encoding DPD (DPYD) are known to be related to severe and lethal fluoropyrimidine toxicity[5].

Not all severe fluoropyrimidine adverse events can be explained by DPYD variants; sometimes, carriers of a DPYD variant associated with an increased risk of severe adverse events may tolerate well the treatment with fluoropyrimidine and may not experience major toxicity[6]. As such, the intervention of other factors capable of affecting DPYD expression may influence fluoropyrimidine metabolism in patients both with and without DPYD variants.

MicroRNAs (miRNAs) are endogenous, short non-coding RNA molecules, about 20-22 nucleotides in length, which are found in all eukaryotic cells and assist in regulating post-transcriptional gene expression by binding to 3'untranslated regions or 5'untranslated regions of target messenger RNAs. This also leads to the inhibition of translation of messenger RNA degradation[7,8]. Almost 30% of human genome proteins are regulated by miRNAs, with half of these genes being associated with cancer[7,8]. MicroRNAs regulate the gene expression involved in essential biological processes such as cell proliferation, cell differentiation, apoptosis, metastasis, and immune response[9-11]. Many studies have tried to prove that miRNAs can be used as diagnostic tools, predic-

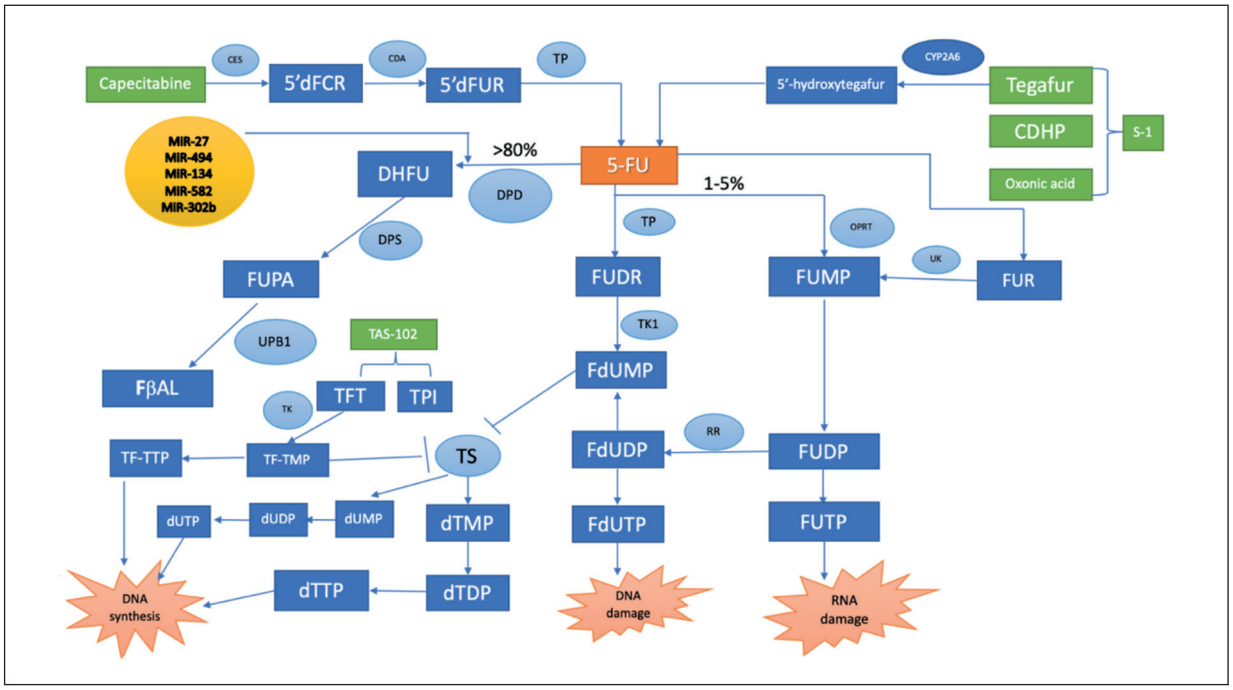

Figure 1. Fluoropyrimidine metabolic pathways

tive biomarkers of disease progression, prognostic biomarkers, survival rate, progression-free survival, treatment responsiveness, and chemotherapy-related toxicity[12-14]. The importance of microRNAs regarding chemotherapy toxicity is less well known.

In our review, we summarize the role of miRNAs in fluoropyrimidine toxicity, the relation with DPD, and how miRNAs may be a tool in selecting patients who will benefit from the efficacy of fluoropyrimidines without developing major toxicities**.**

Fluoropyrimidine-Related Toxicity

Severe toxicity in chemotherapy is usually associated with interruption or treatment discontinuation and often requires hospitalization. Fluoropyrimidine-related toxicity is a well-recognized clinical problem that impacts patients' quality of life and, in some cases, can be life-threatening.

5-FU and Capecitabine can induce grade 3 or 4 toxicity in 10-30% of patients and fatal toxicity in 0.3-2% of patients[15]. When we compare 5-FU to Capecitabine, data from the Adverse Event Reporting System (AERS) of the Food and Drug Administration (FDA) have shown that certain side effects are more common for one or the other, such as myelosuppression has been shown to be more common in patients receiving 5-FU and diarrhea or hand-foot syndrome in patients receiving Capecitabine[16]. The lower prevalence of adverse effects of S-1 and TAS-102, compared to 5-FU and Capecitabine, is due to them carrying a DPD inhibitor in their composition, which increases the bioavailability of 5-FU[3].

5-FU has a narrow therapeutic index; therefore, there is a small limitation between efficacy and toxicity[17]. Adverse events are likely the results of genetic variation, especially ones such as DPD. Decreased DPD activity implies a decreased clearance and an increased half-time of 5-FU, resulting in an increased risk of toxicity[18,19]. More than 80% of the 5-FU dose is converted to inactive metabolite 5-fluoro-5,6-dihydrouracil by DPD[19]. DPD deficiency accounts for 20-60% of fluoropyrimidine-related severe toxicity, and 20-30% can be explained by DPYD deleterious variants[19,20]. It is estimated that 3-5% of Caucasians are carriers of a partial DPD deficiency and that 0.1-0.3% present complete deficiency[20]. Overall, 5% of the general population present a DPD deficiency[21]. This pharmacogenetic "DPD syndrome" manifests as mucositis/stomatitis, myelosuppression, severe diarrhea, and rare events such as acute cardiac ischemia following the first or second cycle of 5-FU, hepatitis, and encephalopathy[22].

DPD expression varies from tissue to tissue and exerts its activity predominantly in the liver, peripheral blood mononuclear cells, and inflammatory and tumor tissues.

The DPD activity is also influenced by the circadian rhythm, which DPD activity follows: DPD activity peaks near midnight, and through DPD activity in the early afternoon; this was discovered during the administration of continuous 5-FU infusions[23].

The activity of DPD has high interindividual variability, but the most important are variants of the DPYD gene, the gene that encodes the DPD enzyme[24].

The DPYD gene allelic variants most frequently associated with toxicity during the treatment with fluoropyrimidine are DPYD*2A (IVS14+1G>A, c.1905+1G>A), DPYD*9B (c.2846A>T), DPYD*13 (c.1679T>G), HapB3 (c.1129-5923C>G)[24-28].

In addition to DPYD gene polymorphisms, polymorphisms of other genes such as thymidylate synthase (TYMS), methylenetetrahydrofolate reductase (MHTFR), enolase superfamily member 1 (ENOSF1), ATP-binding cassette subfamily B member 1 (ABCB1), and cytidine deaminase (CDA) appear to be involved in fluoropyrimidine toxicity[29-34].

Fluoropyrimidine toxicity, especially of 5-fluorouracil, can be manifested through hematological non-cumulative toxicity most common in bolus infusion (neutropenia, thrombocytopenia, anemia), immediate digestive toxicity (nausea, vomiting, diarrhea, stomatitis, ileitis), alopecia in case of continuous perfusion, thrombophlebitis and photosensitivity along the vein pathway, neurological toxicity from high doses (cerebellar ataxia), ophthalmological toxicity through tear excretion (conjunctivitis, tear hypersecretion), skin toxicity usually aggravated by sun exposure (hand-foot syndrome, hyperpigmentation, rash, hives) and reversible cardiac toxicity caused by continuous perfusion (angina, myocardial infarction, myocardial necrosis, coronary dissection, heart failure, arrhythmia, cardiogenic shock, cardiac arrest, and sudden cardiac death)[35-38]. Severe adverse events may appear within the first chemotherapy cycle – a fact that supports the importance of treatment personalization before treatment initiation[37].

Fluoropyrimidine cardiotoxicity represents one of the most severe fluoropyrimidine-induced toxicities. The incidence ranges from 1% to 18%, and chest pain is the most frequent symptom[36,37]. The incidence variability is due to various manifestations of cardiotoxicity, but the risk is influenced by the schedule and route of administration of 5-FU, the use of concurrent chest radiotherapy or anthracycline administration, underlying coronary artery disease, and how frequently the patients were monitored[36,37]. Several mechanisms for fluoropyrimidine cardiotoxicity have been proposed, such as (1) vasoconstriction, (2) direct myocardial injury with systolic dysfunction, (3) accumulation of alpha-fluoro-beta-alanine (FBAL), a metabolite of 5-FU, which is first converted to fluoroacetate and later to fluorocitrate leading to citrate accumulation and downstream depletion of ATP resulting in ischemia, (4) dysfunction of vascular endothelium, (5) hypercoagulable status leading to thrombosis, (5) changes in the shape of erythrocyte membrane leading to increased blood viscosity and decreased ability to carry and release oxygen, and (6) an acute coronary syndrome caused by an allergic reaction also known as Kounis syndrome[3]. 5-FU and Capecitabine have the same frequency of cardiac adverse events, while on the other hand, TAS-102 is considered to be a "cardio-gentle" fluoropyrimidine. The following mechanism seems to be responsible for the lack of TAS-102 cardiotoxicity: (1) trifluorothymidine (TFT), an analogue of thymidine and one of the components of TAS-102, induces a higher level of cell death and does not obtain an autophagic survival response in cancer cell lines, (2) a different oncological target – DNA synthesis, (3) the incorporation of the molecule into the DNA of tumor tissues is higher than the incorporation into DNA of normal tissues, thereby sparing the cardiac tissue, and (4) TAS-102 is not catabolized by DPD, which consequently means a reduced formation of FBAL and a lower risk of cardiotoxicity due to FBAL accumulation[39]. A reduced level of cardiotoxic metabolites also explains the lack of cardiac toxicity of S-1 due to the presence in its composition of gimeracil, a DPD-inhibitor.

How to Assess Fluoropyrimidine Toxicity?

Over time, many studies have focused on studying the mechanisms involved in fluoropyrimidine toxicity. Most strategies have tried to determine the DPD deficiency through genotyping and phenotyping method (Table I).

The fact that DPD activity can affect 5-FU efficacy through the development of important adverse events was demonstrated in many studies, and therefore an upfront screening for functionally relevant DPYD genes and treatment adjustment in patients with related toxicity allelic variants to avoid severe toxicity is justified. The first functional mutation related to fluoropyrimidine toxicity was DPYD*2A. Deenen et al[40] demonstrated in a prospective study the clinical validity and utility of DPYD*2A genotype-guided dosing[40]. However, due to the low frequency of DPYD*2A, less than 1% in Caucasian patients, attention was focused on identifying other DPYD variants associated with DPD deficiency[41]. Until now, four variants have demonstrated their utility and clinical validity based on high-level evidence from meta-analyses[42].

There are situations in which the DPD deficiency is not explained by the presence of DPYD variants or by phenotypic methods. These cases are in part explained by a variability in the regulation of DPD at the post-transcriptional level by microRNA. The 3'-untranslated region of DPYD is directly targeted by mir-27a, miR-27b, miR-134, miR-494, miR-582-5p, and in the cod-

Table I. Available methods to identify patients at risk for severe fluoropyrimidine toxicity[24-28,42-44,48-53].

Stategies	Methods	Evidence
Phenotyping methods	DPD activity in PBM's	Clinical validity established Clinical utility in research
	Endogenous uracil serum concentration	Clinical validity and utility established
	2-^{13}C uracil breath test	Clinical validity and utility in research
	Uracil dose test	Clinical validity and utility in research
Genotyping methods	DPYD mutation	Clinical validity and utility for DPYD*2A, 13, 9B and HapB3
	miRNA mutation	Clinical validity and utility in research

DPD – dihydrodioyrimidine dehydrogenase, DPYD – dihydrodioyrimidine dehydrogenase gene, miRNA – microRNA.

ing sequence by hsa-miR-302b-3p[40-43]. Of these, miR-27-a and miR-27-b have been shown to downregulate DPD expression by targeting an RNA-induced silencing complex (RISC) protein to DPYD[43]. Moreover, miR-27-a functions as an oncogene, and its overexpression has been associated with poor prognostics, increased risk of metastasis and disease progression, and chemotherapy resistance[43].

The clinical relevance of miR-27a polymorphism has been demonstrated in three studies on patients undergoing fluoropyrimidine-based chemotherapy. Amstutz et al[42] showed, in a study with 514 patients who presented fluoropyrimidine-related toxicity after the first two cycles, an association between rs895819 variant G allele of miR-27a and increased early-onset toxicity and also the fact that this association is dependent on the DPYD variant related to DPD deficiency[42]. In the same study, another polymorphism, rs11671784, was analyzed, but no association with fluoropyrimidine toxicity was observed and no significant relation with DPYD mutated variants was detected[42]. Meulendiks et al[43] confirmed the above information in a study including 1592 patients in treatment with fluoropyrimidine[43]. They showed that rs895819 polymorphism is moderately associated with toxicity, but those who have DPYD mutation also have a significantly increased risk grade 3 or 4 toxicity, these facts suggesting the additional role and importance of miR-27-a polymorphism[43]. The carriers of rs11671784 polymorphism showed no significantly increased risk of toxicity regarding the DPYD status[43]. In a small study of 64 patients, Falvella et al[44] showed in both univariate and multivariate analysis the association between rs895819 and grade 3 or 4 adverse events[44].

Another miRNA that shows promising results based on in vitro studies is miR-494. This miRNA, located in chromosome 14q32.31, works as an oncogene in breast cancer metastasis and is overexpressed in hepatocellular carcinoma cells, although some studies also suggest its tumor suppressor role by repressing the proliferation, migration, and invasion of prostate cancer cells by suppression of C-X- C chemokine receptor 4 (CXCR4) gene, and of gastric cancer cells by targeting c-myc[45,46]. Chai et al[45] showed in their study that miR-494 has tumor suppressor function and can sensitize colon cancer cells to 5-fluorouracil by binding to the 3'-untranslated region of DPYD and negatively regulate DPYD expression in modified colon cancer cell lines sensitive to 5-fluorouracil, SW480, and SW480/5-FU cells[45]. So be these meanings, DPYD expression was under the control of miR-494, and 5-fluorouracil resistance and toxicity can be associated with abnormal downregulation of miR-494[47].

The gene encoding for thymidylate synthase (TYMS) has been investigated to highlight the possible role as a biomarker for fluoropyrimidine toxicity and effectiveness. Sinicrope et al[46] revealed that the overexpression of TS in colorectal cancer was related to poor response to 5-fluorouracil, chemoresistance, and toxicity[47]. The TYMS variants associated with fluoropyrimidine toxicity involve tandem repeats of a 28-base-pair sequence instead of three in the 5'-untranslated region or a 6-base-pair variation in the 3'-untranslated region of TYMS, also known as TYMS enhancer region (TSER)[47,48]. The activity of TYMS can also be controlled by microRNA. Increased expression of miR-218 suppresses TYMS-enhanced fluoropyrimidine toxicity and is associated with progression-free survival and overall survival in colorectal cancer[48].

Regarding phenotyping methods, the main methods related to fluoropyrimidine treatment are the determination of DPD activity in peripheral blood mononuclear cells (PBM), uracil dose test, pretreatment determination of the endogenous concentration of dihydrouracil and uracil in plasma, urine, and saliva (UH2/Ura ratio), 2-13C uracil breath test[49-54]. Among all phenotypic methods, the highest accuracy is assigned to pretreatment serum uracil determination[54].

Discussion

Side effects are one of the factors that limit the efficacity of chemotherapy, and they usually require either dose reduction or delayed administration of treatment until remission.

Due to the multiple indications that fluoropyrimidines have, they are probably the most commonly used chemotherapeutic agents, and therefore it is essential to find ways to prevent toxicities that can decrease the efficacity or interrupt the treatment, or which endanger the patient's life.

Many detection methods are available nowadays, phenotypic or genotypic, and we know that the onset of toxicity is influenced by DPD activity. At the present time, four variants of DPYD have proven their clinical utility and their influence on DPD deficiency, but due to the low

frequency and the multitude of mutant variants, their routine testing has not yet progressed into clinical practice. It is estimated that almost 3-15% of patients present a partial DPD deficiency and 0.1-5% a complete deficiency[54].

The Group of Clinical Pharmacology in Oncology (GPCO)-UNICANCER and the French Network of Pharmacogenetics (RNPGx) recommend the following regarding the treatment with fluoropyrimidine: (1) the screening of DPD deficiency before starting treatment with 5-fluorouracil or Capecitabine, (2) performing DPD phenotyping by determination of plasma uracil concentrations and DPD genotyping (variants DPYD*2A, DPYD*13, DPYD*9, and Haplotype B3), (3) reducing the first cycle dose of fluoropyrimidine according to DPD status and later in the treatment to consider increasing the dose according to each tolerance[55].

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has formulated a guideline for better interpretation of clinical DPD genotyping tests and how to adjust the treatment. Based on DPYD genotype and phenotype they calculated DPYD activity score (DPYD-AS) and made the following recommendations: (1) in patients who are DPYD normal metabolisers with activity score of 2 (AS) and two normal functional alleles a dose modification is not recommended, (2) in DPYD intermediate metabolisers, meaning decreased DPD activity and increased risk of toxicity with AS 1 or 1.5, and one normal functional plus one non-functional allele, or two decreased functional alleles, a reduction of 25 to 50% is recommended (the percentage of dose reduction depends on activity score) and (3) in DPYD poor metabolisers with complete DPD deficiency and very increased risk of toxicity, with AS 0 or 0.5 and two non-functional alleles or one non-functional plus one decreased functional allele, it is recommended to avoid the use of fluoropyrimidine treatment; if AS 0.5 and there is no other therapeutic option, 5-fluorouracil should be administered with a significant dose reduction and under therapeutic drug monitoring[56].

Another working group, the Dutch Pharmacogenetics Working Group (DPWG), offers some recommendations regarding fluoropyrimidine treatment. In contrast to the CPIC guideline, DPWG also includes Tegafur in their guideline, not only 5-fluorouracil and Capecitabine; they also recommend patients with AS 0 or 0.5 using a phenotyping method to initiate the treatment with a fluoropyrimidine[57]. The Dutch National guideline for colorectal cancer was updated in September 2017 and recommended DPYD genotyping in all patients before initiating treatment with fluoropyrimidine[58,59].

On 13 March 2020, the European Medicines Agency's (EMA) Pharmacovigilance Risk Assessment Committee (PRAC) has recommended testing for DPD deficiency before initiation of fluoropyrimidine-based chemotherapy; patients with complete DPD deficiency should not receive any type of fluoropyrimidine, and those with a partial deficiency can receive fluoropyrimidine treatment with a reduced dose and later in the course of the treatment adjustment of the dose depending on the toxicities associated[60].

In 2015, uridine triacetate (UT, Vistogard) was approved by FDA as an antidote in case of severe fluoropyrimidine toxicities, with a survival benefit in 96% of cases, based on two open-label, single-arm trials[61,62]. Vistogard can be used in the first 96 hours after the last dose of 5-FU or Capecitabine for life-threatening toxicities such as severe neutropenia, diarrhea, or cardiotoxicity unresponsive to drug cessation and antianginal therapy[63].

In recent years, miRs are involved and control many of the processes involved in cancer, but also in efficacy, resistance, and toxicity to treatment. So far, in terms of fluoropyrimidine toxicity, only mir-27 has proven its potential role as a predictive factor. Previous studies[45-47] have illustrated that the presence of rs895819A>G presents only a moderate risk of toxicity, but the association of rs895819A>G and a DPYD variant are associated with an increased risk of early-onset severe fluoropyrimidine toxicity. This fact highlights the important role that microRNAs could play in DPYD genotyping and, subsequently, in providing an appropriate treatment with limited side effects. As mentioned previously, CPIC recommends the association of phenotyping and genotyping for the screening of DPD deficiency, but the combined determination between polymorphism on DPYD gene and microRNA can be a method with greater accuracy and could help us detect more patients who may be suffering from major toxicities.

Conclusions

Even today, when target therapy and immunotherapy, alone or combined with chemotherapy, tend to become the first-line treatment in many

types of cancer, fluoropyrimidine is still the cornerstone in the treatment of many types of cancer and represents one of the most used chemotherapies worldwide. DPD deficiency and its implications in fluoropyrimidine toxicity have been extensively studied over time, and as a result of these studies, the determination of DPD activity before treatment is recommended or even implemented; an example would be the Netherlands and some of the centres in France. EMA reinforces the studies and recommends testing DPD deficiency before starting treatment and adjusting the dose according to the degree of the deficiency.

The role of miRNA in fluoropyrimidine toxicity is still unclear. On the one hand, we need several studies that will confirm the clinical utility of miRNAs that are known to be involved, such as miR-27 or miR-494; on the other, we need studies to identify other new miRNAs to help us easily identify patients who are more likely to suffer more severe adverse effects. Possible directions of research are finding circulating miRNAs independent of DPD variants or demonstrating possible correlations between the concentration of 5-FU or their metabolites and the expression of miRNAs.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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