Proton-coupled folate transporter as a biomarker of outcome to treatment for pleural mesothelioma

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First draft submitted: 7 May 2018; Accepted for publication: 8 May 2018; Published online: 19 June 2018

Keywords: chemoresistance • mesothelioma • PCFT • pemetrexed

The main objective of pharmacogenetics is the identification of genetic features involved in clinically meaningful variations in drug responsiveness. Therefore, pharmacogenetics may reduce the variation in individual response to drugs and for tailoring therapies according to genetic profile, fulfilling the promise of precision medicine [1].

The problem of interindividual variability in drug response is particularly important in anticancer regimens, which are characterized by a narrow therapeutic window. Small alterations in the transport or metabolism of anticancer agents may indeed cause large changes in their pharmacological effects, both in terms of toxicity and efficacy.

As reported by the Pharmacology and Molecular Mechanisms group of the European Organization for the Research and Treatment of Cancer, the terms ‘pharmacogenetics’ and ‘pharmacogenomics’ are often used interchangeably and pharmacogenetics is considered as concerning the individual patient’s characteristics and pharmacogenomics those of the tumor [2]. However, according to this position paper as well as to the definition by the European agency for the evaluation of medicinal products ‘pharmacogenetics’ focuses on the association of one gene or several genes with drug activity, while ‘pharmacogenomics’ considers the whole genome, through the broader application of new genomic technologies [3]. Thus, in oncology, a pharmacogenetic approach aims to customize the chemotherapy treatment according to individual/somatic as well as tumor genetic characteristics. This represents a modern and intriguing challenge and pharmacogenetic tools are warranted to maximize the therapeutic efficacy and minimize useless treatments, especially in patients affected by solid tumors with limited therapeutic approaches, such as malignant pleural mesothelioma (MPM).

MPM is an aggressive tumor arising from the pleura, with grim prognosis. Its incidence is increasing throughout most of the world and it is predicted that it will rise in the next 10 years [4]. Most patients with MPM are not amenable to radical surgery and systemic therapy is the only potential treatment option. A number of prognostic factors have been described and are part of two prognostic scoring systems. In the European Organization for the Research and Treatment of Cancer score, poor prognosis is associated with a poor performance status, a high white blood cell count, male gender and having sarcomatoid histologic subtype, while In the Cancer and Leukemia Group B scoring system pleural involvement, LDH >500 IU/l, poor PS, chest pain, platelets >400,000/μl, nonepithelial histology and age older than 75 years, jointly predict poor overall survival (OS).

Based on extensive genomic profiling, four MPM molecular subtypes were associated with OS [5]. However, up to now, there are no data about specific genetic biomarkers of response to chemotherapy in MPM patients. The identification of such molecular predictors is urgently needed in order to select patients for optimal treatment strategies and to improve clinical outcome.

More than 14 years ago, US FDA approved the first ever (and the last so far) treatment drug to be used in the first-line treatment of MPM in combination with cisplatin, pemetrexed. In fact, this combination significantly improved the response rate (41.3 vs 16.7%; $p < 0.0001$), time to progression (5.7 vs 3.9 months; $p = 0.001$), OS (12.1 vs 9.3 months; $p = 0.020$) and quality of life compared with cisplatin alone. The combination with carboplatin gave similar results [6].

Pemetrexed is a multitargeted antifolate agent that enters the cancer cells through different transporters and is then converted to a series of analogous polyglutamate derivatives by the enzyme folylpolyglutamate synthetase. This polyglutamylation leads to extended intracellular retention, resulting in more prolonged efficacy. Active metabolites of pemetrexed inhibit several folate-dependent enzymes such as thymidylate synthase, dihydrofolate reductase, glycylamide ribonucleotide formyl transferase and to a lesser extent, aminoimidazole carboxamide ribonucleotide tranformylase and CI-tetrahydrofolate synthase, thereby inhibiting both pyrimidine and purine biosynthesis [7].

In vitro studies demonstrated that several factors increase the antitumor activity of pemetrexed, including low expression levels of the main target TS, high activity of folylpolyglutamate synthetase and the rapid transport across the plasma membrane mediated by influx transporters for which pemetrexed has different affinities [8]. In agreement with previous data in nonsquamous and squamous specimens from lung cancer patients treated with pemetrexed-based chemotherapy, as reviewed by Santarpia and collaborators [9], high TS expression has also been associated with poor response, as well as significantly shorter median progression-free survival and OS in the two largest multicenter studies on pemetrexed-based regimens in MPM [10,11].

In a more recent translational study, the efficacy of pemetrexed was associated with both low TS and high proton-coupled folate transporter (PCFT/SLC46A1) expression levels [12]. In fact, a high mRNA expression of *PCFT* and a low-mRNA expression of *TS* were associated with lower risk of developing a progressive disease compared with disease-control as well as with significantly longer progression-free survival and OS. Low-PCFT protein levels were also associated with shorter OS and multivariate analysis confirmed PCFT-independent prognostic role.

In mammalian cells, three distinct processes mediate membrane transport of folates and antifolates, including pemetrexed [13]. The reduced folate carrier (RFC) is a carrier-mediated anion exchanger, facilitating the translocation of a folate substrate across the cell membrane by the co-transport of another anion in the opposite direction. With a K_i for methotrexate influx ranging from 2 to 5 $\mu\text{mol/l}$ in murine and human tumor cell lines, RFC has a higher affinity for methotrexate than for folic acid. Of note, the affinity for pemetrexed is twofold higher [14]. The folate receptors FR- α and FR- β are binding proteins anchored to the cell membranes by a glycosyl phosphatidylinositol tail that mediates transport by an endocytotic process. The maximum rate of transport into cells mediated by this mechanism is 100, the rate mediated by RFC. Hence, FR-mediated transport contributes little to the uptake of pemetrexed. The third folate transport system, characterized by a unique and distinct low-pH optimum that is present in the majority of human solid tumors, is mediated by PCFT. This transporter was initially characterized in mice, and only recently emerged as the main transporter mediating pemetrexed influx, with remarkable transport k_m values of 0.2–0.8 μM [15].

The pivotal role of PCFT in the transport of folates and antifolates has been demonstrated in various models, showing that PCFT transfection in HeLa cells increased pemetrexed cytotoxicity [16], while PCFT silencing increased IC_{50} values by fourfold and threefold in the MSTO-211H and H2452 MPM cells, respectively [12]. Of note, PCFT promoter can be silenced via DNA methylation [17], resulting in reduced transcriptional activity. Indeed, H28 MPM cells, which displayed methylation of 85% of the CpG-islands of *PCFT* promoter had low-expression levels of PCFT and were relatively more resistant to pemetrexed [12]. Additional experiments were then performed to evaluate PCFT expression and cell proliferation after DNA demethylation. These experiments were performed with 5-Aza-2'-deoxycytidine, which significantly reduced the methylation of the *PCFT* promoter, down to -70% in the H28 cells and subsequently increased the mRNA expression of *PCFT* as well as the growth inhibitory activity of pemetrexed. This means that PCFT was not only identified as a new biomarker that can help predict pemetrexed's effectiveness or chemoresistance, but also a potential useful target to overcome such chemoresistance.

Where do we go from here? We suggest the following rational preclinical and clinical development strategies. First of all, additional preclinical studies in appropriate *in vivo* models of MPM should evaluate the key role of PCFT in the antitumor activity of pemetrexed and other potential molecular mechanisms underlying the differential PCFT

expression, such as, for instance, the hypoxic/metabolic status of these tumors, which has also been correlated to pemetrexed resistance [18] and might suggest novel antiglycolytic therapeutic strategies [19,20]. Second, translational studies with prospectively collected samples, are essential for the validation of the previous retrospective data as well as for the identification of the best cut-off expression level for a validated pharmacogenetic test. Finally, a Phase I basket trial should test 5-Aza-2'-deoxycytidine, which is a well-tolerated compound currently used to treat myelodysplastic syndrome, in patients with different tumor types, such as mesothelioma and lung cancer, to be treated with pemetrexed and cisplatin and stratified according to PCFT expression. Subsequently, Phase II and Phase III trials could then select the best tumor type and establish the role of this new potential pharmacogenetic-guided treatment versus the standard of care backbone chemotherapy, according to previously standardized and validated thresholds.

Hopefully, the results of these studies should enable oncologists to stratify patients based on PCFT before anticancer treatment, allowing the optimization of clinical outcomes through effective personalization of treatment.

Financial & competing interests disclosure

This work was partially supported by 'the Law Offices of Peter G Angelos Grant' from the Mesothelioma Applied Research Foundation (MARF), USA. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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