Epigenetic modulation of resistance to chemotherapy?

Drug sensitivity is influenced by alterations in gene expression [1]. Gene expression patterns in tumours are determined by both genetic and epigenetic changes [2]. Epigenetic changes are stable and heritable changes in gene expression resulting from modifications of DNA and associated proteins that do not involve an alteration in DNA sequence. Epigenetic modification occurs both during tumour development and during the acquisition of drug resistance and leads to altered expression of many hundreds of genes [3]. Unlike genetic changes, epigenetic modifications require active maintenance so they can be manipulated by small molecules and provide a potential therapeutic target [4]. Two common epigenetic changes are CpG island methylation and histone acetylation, both of which can be modulated using DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors, respectively. DNMT inhibitors are able to reverse gene silencing associated with promoter methylation and this is enhanced in vitro by the combination of DNMT and HDAC inhibitors [5].

Epigenetic therapies such as DNMTi and HDACi have shown considerable promise in the treatment of haematological malignancies [4]. Their ability to inhibit growth of solid tumours is less clear. Clinical trials of DNMT inhibitors such as 5-azacytidine and 2′-deoxy-5-azacytidine have been ongoing for >30 years ago, but initial studies in solid tumours showed little clinical activity [6]. However, clinical trials of such epigenetic therapies are now being re-evaluated as a result of our increased understanding of their potential mechanisms of action.

Recent trials of DNMT inhibitors in haematological malignancies have shown that greater clinical responses are obtained at doses substantially less than the maximum tolerated dose used in earlier studies [7]. This parallels in vitro studies of DNMT inhibitors done many years before which showed that maximal demethylation occurred at lower doses, while at high doses cell death and inhibition of growth occurred due to the DNA damaging rather than the epigenetic effects [8]. Pharmacodynamic markers are now available that can be used to direct the use and scheduling of these agents, which may be particularly important when combining them with cytotoxic chemotherapy [4]. The combination of DNMTi and cytotoxics such as cisplatin have previously been examined with disappointing results [9], but recent pharmacodynamic studies of the kinetics of demethylation suggest that the scheduling of these agents would not have been appropriate to obtain the maximum effects of demethylation on gene expression at the time of administration of the cytotoxic agent [10].

It is possible that there are subsets of patients with certain tumour characteristics who will particularly benefit from epigenetic therapies and if so, it will be important to investigate molecular biomarkers (such as methylation profiling) that might identify such patients. Investigation and validation of these predictive biomarkers in early trials may allow subsequent trial populations to be enriched for patients with greater likelihood of benefit, thereby increasing the power of the trials to observe an effect. There is, therefore, a need to re-evaluate epigenetic therapies in solid tumours using our increased understanding of their mechanism of action to assess their efficacy in more appropriate trial designs that include epigenomic profiling.

Candelaria et al. [11] have examined whether the addition of a combination of HDAC and DNMT inhibitors in patients with disease progressing on standard therapies is able to reverse drug resistance and halt progression. This is based on the hypothesis that epigenetic inactivation of drug sensitivity genes has occurred in these tumours and that reactivation of these genes by the epigenetic drugs will sensitise the tumours to chemotherapy. This is a nonrandomised trial where patients with a range of tumour types, progressing on a variety of chemotherapy regimens, were treated with the combination of hydralazine, a weak DNMTi and magnesium valproate, an HDACi, starting 1 week before re-initiation of their previous chemotherapy. Their results are certainly provocative. Of 17 eligible patients that received study intervention, 12 had clinical benefit (four partial response + eight stable) following introduction of hydralazine and magnesium valporate despite previous progression. However, the results should be interpreted with caution. The study population was very heterogeneous with different tumour types and different regimens including hormonal therapy. Progression was defined by Response Evaluation Criteria in Solid Tumors (RECIST) criteria but it in not clear whether this was independently verified or what time interval was allowed from time of progression to the start of protocol therapy. The population included was already a selected population since those with early progression during the first week of hydralazine and magnesium valproate were excluded. Three of the responses were seen in ovarian cancer patients and defined by GCG CA125 criteria. It would be useful to know whether the responses by RECIST criteria were consistent with the CA125 criteria as the effect of combined HDAC and DNMT inhibition on CA125 expression is not known. Nevertheless, it is intriguing that reversal of resistance might be possible with a relatively well-tolerated treatment and this is a concept that is being tested in a number of ongoing clinical trials of epigenetic therapies. As these studies are developed, it is important that...
pharmacodynamic markers of activity are investigated and validated in order to support and substantiate early clinical data and inform decisions about which agents and schedules to take on for further investigation.

As with other previous studies [9, 12], Candelaria et al. examined activity of each compound in the surrogate tissues of peripheral blood: 5-methylcytosine levels for hydralazine and histone deacetylase activity for valproate. Surprisingly, variable levels of methylation are observed for patient’s pretreatment, making it difficult to interpret for individual patients the variable methylation observed after chemotherapy. Overall, a significant \( P = 0.048 \) decrease in methylation is observed after treatment, although this is perhaps not as robust or as reproducible as obtained for nucleoside DNMT inhibitors [9, 12]. Inhibition of HDAC activity is also observed, although histone acetylation per se, the most widely used pharmacodynamic marker of HDAC inhibitors, has not been examined in this surrogate tissue, therefore it is more difficult to make a comparison with previous studies using other HDAC inhibitors.

Many tumours release DNA that can be detected at low levels in body fluids. However, this is very variable. Studies that have examined whether the same genetic or epigenetic changes are present in serum or plasma DNA as are present in the corresponding tumour report 20%–70% specificity [13]. Theoretically this approach has the potential to allow a relatively noninvasive means of monitoring demethylation in tumour DNA before and after chemotherapy. However, the low specificity means that caution must be used in interpreting these data. Currently, there is no robust means of quantifying the amount of tumour DNA rather than nontumour DNA present in body fluids. So disappearance of methylation as detected by methylation-specific PCR may be due to demethylation of the tumour DNA by the DNMT inhibitor or due simply to there being no tumour DNA present after treatment. This will be particularly compounded if the tumour is responding to chemotheraphy.

Endpoints that assess whether a pharmacodynamic target is being affected such as demethylation in peripheral blood or histone deacetylation in peripheral blood mononuclear cell are important and provide a first step in evaluating a potential epigenetic therapy. However, markers of biological effect such as markers of gene re-expression or induction of cell death may be more informative about efficacy and therefore be more useful in determining optimal schedules and drug combinations. Identification of markers that predict for response to treatment is also a high priority, but their sensitivity, specificity and reliability need to be very carefully evaluated before they should be used to determine future trial design. As these pharmacodynamic and biomarker endpoints are more widely used, it will be vital to standardise and harmonise the methodology in a quality assured manner. This will allow more sophisticated comparison of different treatment regimens at an earlier stage and more confidence about the design of the eventual definitive randomised phase III trial. We would argue that randomised phase II trials with biological endpoints included as trial objectives, which assess pharmacodynamic efficacy and kinetics of the drugs and begin to evaluate the potential of predictive markers, will become increasingly important.

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