Nature Reviews Cancer | AOP, published online 13 September 2013; doi:10.1038/nrc3605

EPIGENETICS

Showing a more sensitive side

The long-term disease-free survival of patients with diffuse large B cell lymphoma (DLBCL) treated with the rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) regimen is 65%. However, for patients who relapse or have refractory disease the outlook is bleak. Leandro Cerchietti, Ari Melnick and colleagues present evidence that DNA methyltransferase inhibitors (DNMTIs) might be one route through which to improve the outcome for patients with refractory disease.

DLBCL cells have an aberrant methylation pattern and rapid proliferation, prompting the authors to examine the effect of a DNMTI, decitabine, on cell viability and chemosensitization. Examination of a panel of 30 DLBCL cell lines identified eight cell lines that had a decreased level of 5-methylcytosine and that died by apoptosis in response to low concentrations of decitabine. Moreover, the growth of one of these lines as a xenograft in immunocompromised mice was suppressed by treatment with decitabine.

> “prolonged pretreatment with a DNMTI is important to induce chemosensitivity in chemoresistant cells”

PHOTODISC

Four of the DLBCL cell lines that were sensitive to decitabine were also sensitive to doxorubicin (one of the R-CHOP regimen drugs). The combined use of decitabine and doxorubicin induced synergistic cell killing in all four cell lines and, when compared with either drug alone, combined use more effectively suppressed the growth of xenografts established from one of the four cell lines in mice. Therefore, low-dose decitabine alone can induce methylation changes that are lethal in a minority of sensitive cells and can synergistically increase the sensitivity of these cells to doxorubicin. What about the majority of DLBCL cell lines (those that the authors found were resistant to cell death induced by either decitabine or doxorubicin)?

The authors exposed two cell lines established from patients with refractory DLBCL to prolonged (5-day) treatment with low-dose decitabine. The cells underwent DNA hypomethylation and showed a reduced rate of proliferation, along with evidence of senescence. Senescent cells are known to be sensitive to doxorubicin, and exposure of these cell lines to decitabine 5 days before treatment with doxorubicin substantially increased their sensitivity to this chemotherapeutic drug. Importantly, significant suppression of the growth of xenografts established from one of these cell lines was only evident when decitabine was administered for 10 days followed by treatment with doxorubicin, indicating that prolonged pretreatment with a DNMTI is important to induce chemosensitivity in chemoresistant cells.

To better understand the mechanisms at work in chemosensitization by DNMTIs, the authors analysed gene expression and carried out DNA methylation profiling in chemoresistant and chemosensitive DLBCL cell lines; of the nine genes reproducibly hypermethylated in chemoresistant cell lines, the authors further investigated *SMAD1* because of its known effect in chemotherapy-induced senescence. In chemoresistant DLBCL cell lines exposed to decitabine for 5 days, the level of *SMAD1* hypermethylation was reduced and the *SMAD1* transcript level increased. Why does an increase in *SMAD1* expression sensitize cells to chemotherapy? The authors showed that increased levels of SMAD1 protein were associated with a re-establishment of growth arrest induced by bone morphogenetic protein or transforming growth factor-β.

The authors applied their new knowledge to the clinic and treated 12 patients with the DNMTI azacitidine at escalating doses for 5 days before the standard R-CHOP regimen. Although 11 of these 12 patients were judged to be at a high risk of relapse, 11 of the patients achieved a complete response and ten of the patients remained in remission at a median follow-up of 13 months. Data from six of the patients indicated a reduction in the methylation of *SMAD1* after treatment with azacitidine. Additionally, a comparison of chemosensitivity in cells established *ex vivo* from one of the patients before and after treatment with azacitidine showed an increase in chemosensitivity only after treatment. The authors hope that their data will lead to bigger trials to further develop the strategy of using epigenetic-based therapies to overcome chemotherapy resistance.

Nicola McCarthy

ORIGINAL RESEARCH PAPER Clozel, T. *et al.* Mechanism-based epigenetic chemosensitzation therapy of diffuse large B-cell lymphoma. *Cancer Discov.* http://dx.doi.org/10.1158/2159-8290.CD-13-0117 (2013)