

Lack of scientific evidence for resistance to perforin-dependent cytotoxic pathways in lymphomas: no justification for skepticism for search of effective immunotherapy against lymphomas

Rationale

The failure of Wilms tumor 1 antigen (WT1)–specific cytotoxic T lymphocytes CTLs to kill lymphoma has been attributed to resistance to perforin (prf.).¹ Perforin-dependent cytotoxic pathways or cytotoxicity mediated by cytotoxic granules represent the major cytotoxic pathways utilized by CTLs and Natural Killer (NK) cells to induce tumor cell death. Perforin plays an indispensable role in the delivery and activation of granzymes in target cells, whereas granzymes are diversified with different activities leading to target cell death.²

Various experimental studies suggest that these pathways are disturbed in lymphoma and other malignancies. Expression of proteinase inhibitor–9 (PI-9), a molecule that selectively inactivates granzyme B (grzB.), is considered an immune escape mechanism in lymphomas.^{3,4} Ectopic expression of PI-9 in human malignancies has been considered to represent the major factor determining the effectiveness of immunotherapeutic approaches.³ Furthermore,

overexpression of antiapoptotic molecule B-cell leukemia/lymphoma 2 proteins (bcl-2) known to inhibit cytotoxicity mediated by purified prf./grzB. has been found in a significant proportion of lymphomas.⁵ Despite the potential relevance of the expression of PI-9 and bcl-2 in determining the sensitivity of lymphoma to cell-mediated lysis, no functional studies have been reported in human lymphoma.

Project report

We modified our primary project following reports indicating a high prevalence of resistance to perforin-dependent pathways in primary lymphomas and possibly in other tumors detected by using WT1-specific CTLs in vitro, which could critically determine the clinical efficacy of WT1-specific T cell-based treatment approaches¹.

We investigated the expression of the PI-9 molecule and over-expression of the bcl-2 molecule in lymphoma cell lines and primary lymphoma cells. We found PI-9 expression and bcl-2 over-expression in a significant proportion of cell lines and primary lymphoma cells. Subsequently, we investigated the correlation between expression of both molecules in lymphomas and their susceptibility to cytolysis mediated by specific CTLs and allogeneic NK cells.

All primary lymphoma cells and cell lines were sensitive to cytolysis by CTLs and cytokine-activated NK cells, and no difference in sensitivity was observed with respect to PI-9 expression and/or bcl-2 overexpression. Cytolysis was mediated predominantly through perforin-dependent pathways. In contrast, the vast majority of lymphoma cell lines and primary lymphoma cells was resistant to cytolysis mediated by resting allogeneic NK cells. This resistance, however, was related to inability of lymphoma cells to induce degranulation of resting NK cells. Detailed results of our study were published in the journal Blood.⁶

Conclusion

Our study has shown that primary expression of PI-9 with or without bcl-2 overexpression in human lymphomas did not confer resistance to cytotoxicity mediated by immune effector cells in vitro. Thus, our findings warrant the conclusion that lymphomas are sensitive to perforin-dependent cytotoxic pathways despite expression of PI-9 molecules and overexpression of bcl-2 molecules. This conclusion is justified despite the fact that expression of both antiapoptotic molecules in tumor cells is known to confer resistance to cell death mediated by purified prf./grzB. This granzyme B resistance must be considered with respect to the redundant or dispensable role of grzB. and with respect to the quantitative expression of both antiapoptotic molecules PI-9 and bcl-2 in tumor cells.

Our interpretation is based on comprehensive in vitro and in vivo studies with knock-out mice reported by Trapani's group investigating a selective role of prf., grzA. and grzB. in immune-mediated eradication of lymphomas.⁷ Lymphoma cells grew efficiently in perforin-deficient mice, whereas granzyme A- and/or B-deficient mice rejected large tumor doses as avidly as wild-type mice, indicating that grzB. was completely dispensable or redundant and can be substituted by other granzymes in NK/CTL-mediated lymphoma eradication via perforin-dependent pathways. These in vivo experiments correlated with in vitro experiments. Granzyme B-deficient (grzB^{-/-}) murine lymphocytes retained

potent cytolytic activity in vitro in contrast to perforin-deficient (pfp^{-/-}) lymphocytes that displayed nearly no cytolytic activity.⁸

Three clinical studies that investigated the association of ectopic PI-9 expression in tumor tissues with clinical outcome resulted in considerable controversy. Two initial studies reported an adverse effect of PI-9 expression on survival in anaplastic B-cell lymphoma⁴ and progression-free survival in melanoma.⁹ Interpretation of both studies is doubtful as none used clinically effective immunotherapy in which granzyme B resistance might play a role. Both studies sharply contrast with a recently published study by Bossard et al.¹⁰ showing significantly better overall survival of patients with PI-9 positive compared to PI-9 negative nasal NK/T-cell lymphomas treated with standard chemotherapy. These results led to the hypothesis that the loss of PI-9 expression reflects dedifferentiation which might be connected with resistance to chemotherapy.

In summary, our study did not confirm the hypothesis that primary ectopic expression of PI-9 with or without overexpression of bcl-2 endows lymphoma cells with resistance mechanisms against NK/CTL-mediated cytotoxicity via perforin-dependent pathways. Therefore, it is unlikely that expression of these antiapoptotic molecules in lymphomas represents a clinically relevant resistance mechanism against immune cell therapy.

One criticism of our study is that stimulation of CTLs with low doses of IL-2 (30 IU/ml), which was used for their expansion, could lead to supraphysiological

stimulation of CTLs and thus underestimate the protective role of antiapoptotic molecules in tumor cells.¹¹ This level of activation is achievable clinically.

Nonetheless, the same reviewer concluded that there is still now no compelling scientific evidence demonstrating the resistance of cancer cells to perforin-dependent pathways.¹¹

References

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