Glutathione S-transferases in bone marrow metastases of disseminated neuroblastoma

A G Hall, A G McGuckin, A D J Pearson, A R Cattan, A J Malcolm, M M Reid

Abstract
Antisera to each of the three main cytosolic forms of glutathione S-transferase (GST; \( \alpha \), \( \mu \), and \( \pi \)) has been used to characterise GST expression by metastatic neuroblastoma in bone marrow trephine biopsies taken from 15 patients at presentation and from five of this group at relapse. There was no correlation between expression of extranuclear \( \alpha \) or \( \mu \) GST and outcome, and no consistent pattern at relapse. Seven of eight expressing nuclear \( \pi \) GST at presentation died of resistant disease. Three of five cases with no detectable nuclear \( \pi \) class GST remain alive and disease free. The results provide no encouragement for further investigation of \( \alpha \) or \( \mu \) GST in this disease but larger studies of uniformly treated patients may show whether nuclear \( \pi \) GST expression at presentation indicates likely relapse.

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Methods
Fifteen patients with disseminated neuroblastoma were studied. The median age was 4 (range 1–18) years. All had bone marrow infiltration at diagnosis. Patients were treated by chemotherapy with vincristine, cisplatin, carboplatin, etoposide, cyclophosphamide, or ifosfamide and high dose melphalan.

Bone marrow biopsy specimens were obtained by trephine as part of routine staging or reassessment. Many patients had multiple biopsies taken at each assessment. Cores (1–2 cm) fixed in 10% neutral buffered formalin and decalcified with Gooding and Stewart’s solution were embedded in paraffin wax. Duplicate 3 \( \mu m \) sections, placed on poly L-lysine treated slides, were prepared for each antibody to be tested, one of which was pretreated with trypsin. Reactivity with antisera to \( \alpha \), \( \mu \), and \( \pi \) GSTs was determined by immunohistochemical techniques as previously described.3 Similarly prepared sections of human kidney were used as positive controls and normal rabbit serum as a negative control.

Sections were independently assessed by two of us (MMR and AGH) without knowledge of the patients’ identities and scored for nuclear or extranuclear positivity by a system in which no staining was designated by 0, moderate staining by +, and heavy staining by +++. In any in which there was disagreement the final score was determined after joint review through a double headed microscope.

Results
Twenty eight biopsy specimens were studied. In 144 of the 168 test slides there was agreement concerning the level of nuclear and extranuclear staining, a concordance rate of 85%. Considerable heterogeneity in staining intensity was found, both between different sites from the same patient and within individual cores. The most positive result obtained for each patient was taken as the final result. Staining was found both within the nuclei and in extranuclear sites, but it was usually difficult to distinguish true cytoplasmic staining of neuroblasts and reactivity with stromal material, whether cellular or extracellular. Trypsinisation had no significant effect. The table shows the patterns of reactivity for trypsinised sections.

Extranuclear \( \pi \) GST was detected in 14 of the 15 patients but it was within the nuclei in
only eight. Nuclear α GST was detected in seven, extranuclear α in one, nuclear μ in 14, and extranuclear μ in nine patients. There was no consistent pattern of differences between samples taken at presentation or at relapse. The table shows the outcome of the 15 patients. There was no relation between extranuclear π GST expression and outcome, as of December 1992. Seven of the eight with nuclear π GST have died of resistant disease. Three of the seven with π GST negative tumour nuclei at presentation remain alive and disease free at 60, 60, and 22 months from diagnosis, and are the sole survivors from this group of 15 patients. Two patients with π GST negative nuclei at presentation died from toxicity and two from disease. On relapse two of five patients had π GST negative nuclei. In both cases nuclei were positive on presentation. All relapsed cases have died of resistant disease.

Discussion

This study shows that cytosolic GSTs, in particular the π class, are often found in bone marrow metastases of neuroblastoma, both at presentation and relapse. Unfortunately they can not be used as markers for the presence of bone marrow infiltration because normal bone marrow cells also express them.

Despite the small numbers of patients these preliminary investigations suggest that detection of extranuclear GSTs will not distinguish between chemosensitive and resistant tumours in patients with disseminated neuroblastoma. Bourhis et al.9 who used northern blotting to assess the level of π GST gene expression, came to a similar conclusion. Recent experiments in which GST genes were transfected into a human breast cancer cell line also suggest that expression of GST does not on its own confer cellular resistance to a wide range of cytotoxic drugs, including some (such as cisplatin) commonly used in the treatment of neuroblastoma.10

Nuclear expression of π GST at presentation, however, might be associated with resistance. The confounding influences of different treatment regimens and the distribution of toxic deaths within the study demand caution in interpretation. Confirmation of an association between nuclear π GST expression at presentation and treatment failure will require larger studies of uniformly treated patients. At present there is no well developed biological rationale for such a link, but nuclear expression of π GST in cervical epithelium has been suggested as a marker of differentiation.1 There was no relation between nuclear π GST expression and response on relapse, suggesting that such expression is not a prerequisite for resistance. As resistance in tumours treated with complex multiagent regimes is likely to be multifactorial this is perhaps not surprising. Further studies of tissues derived from the neural crest, including ganglioneuroblastoma and poor prognosis disseminated neuroblastoma, as well as more common adult tumours, might be of value in the identification of a useful prognostic marker.

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