Laboratory Investigation

Heterogeneity of chemosensitivity in six clonal cell lines derived from a spontaneous murine astrocytoma and its relationship to genotypic and phenotypic characteristics

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Summary

Heterogeneity in drug sensitivity must, in part, account for the relative lack of success with single agent chemotherapy for glioblastoma multiforme (GBM). In order to develop in vitro model systems to investigate this, clones derived from the VM spontaneous murine astrocytoma have been characterised with regard to drug sensitivity. Six clonal cell lines have been tested for sensitivity to a panel of cytotoxic drugs using an intermediate duration 35S-methionine uptake assay. These lines have previously been extensively characterised with regard to morphological, antigenic, kinetic, tumourigenic potential in syngeneic animals and chromosomal properties and display considerable heterogeneity. The present study indicates that heterogeneity extends to sensitivity to all classes of cytotoxic drugs. The greatest difference in sensitivity between the clones was seen in response to cell cycle-specific drugs like the Vinca alkaloids (14-fold and 20-fold for vincristine (VCR) and vindesine (VIND) respectively), while the nitrosoureas, CCNU and BCNU displayed a smaller fold difference in sensitivity (4.3 and 3.6-fold difference respectively). All the clones were considerably more resistant to the adriamycin (ADM), cis-platinum (C-PLAT) and the Vinca alkaloids than the parental cell line although the difference in sensitivity between the clones and parental cell line were less marked for the nitrosoureas and procarbazine (PCB). It has also been possible to examine the relationship between drug sensitivity and the phenotypic and genotypic properties of these clonal cell lines. There is a relationship between chromosome number and sensitivity of a wide variety of cytotoxic drugs including the nitrosoureas, Vinca alkaloids, PCB, C-PLAT, BLEO but not ADR or 5-FU. Clones with small numbers of chromosomes were more resistant than clones with gross polyploidy. Similarly, sensitivity to Vinca alkaloids and ADM, but not other classes of drugs, was greatest in cells with numerous cyttoplasmic processes and which did not express large amounts of cell surface fibronectin. Preliminary experiments have been conducted on reconstituting clonal mixtures of cells with different sensitivity to Vinca alkaloids and results from these studies indicate that the drug resistance phenotype is dominant, with clonal mixtures of sensitive and resistant cell adopting the sensitivity of the more resistant partner. These cell lines should prove to be useful models for examining the cell biological basis of drug resistance in glioma and may lead to the identification and exploitation of novel cellular targets in new therapies for GBM.
Introduction

GBM remains amongst the most devastating and difficult forms of cancer to treat. Despite concentrated basic and clinical research efforts, advances in the treatment of gliomas have been slow. Although GBM responds to adjuvant treatment with chemotherapeutic agents they invariably recur. These failures may be the result of inadequate drug delivery, but may also occur because of the development of drug resistance within the tumour or the overgrowth of resistant clones present in the tumour at the time of diagnosis. Apparent drug resistance may occur because cells are able to effectively repair drug-induced damage [1] or because of the presence of a number of different clones of cells within the tumour, all with differing drug sensitivities [2].

There is considerable evidence that heterogeneity, particularly with regard to chromosomal/DNA content and phenotypic expression, exists within human gliomas [3]. Cell lines derived from the VM spontaneous murine astrocytoma have been used as the basis of an in vitro-in vivo model of GBM [4, 5]. From one of these cell lines VM/Dk P497, six clonal lines have been obtained, which have been shown to express dissimilar morphological, antigen, kinetic and chromosomal properties [6]. We have examined the in vitro chemosensitivity of these clonal lines in order to determine whether the established heterogeneity extends to drug sensitivity.

Although some information is available concerning the mechanisms, at the molecular level, which are responsible for the development of drug resistance in mammalian cells, relatively little attention has been paid to cell biological features which may be related to the predisposition to drug resistance. This paper describes initial observations which relate cell biological features with drug resistance. These studies may have important implications for studies on human gliomas. It is possible that this may provide information of prognostic importance in individual patients or lead to the identification of novel cellular targets in drug resistant glioma cells which can be exploited therapeutically. The development of clonal cell lines with distinct chemosensitivities provides an interesting model system to investigate the therapeutic consequences of cell-to-cell interaction. Much of the pioneering work in this area has been conducted by Heppner and her colleagues (for recent review, see [7]) who have been able to demonstrate that the chemosensitivity and biological behaviour of a wide variety of rodent cell lines can be profoundly affected by the presence of one or more other cell lines in a mixed culture. Studies with chemically-induced rodent glioma cell lines indicate that sensitivity to nitrosoureas can be increased in a sensitive line when grown in contact with a resistant cell line in three dimensional culture, presumably as a consequence of cell-to-cell contact [8]. It is not clear if this is a generalised phenomena in gliomas and what clinical significance this has but data presented in this paper suggests that it occurs in the VM/Dk model system and that cell-to-cell contact modulates sensitivity to Vinca alkaloids as well as nitrosoureas.

Materials and methods

Cell lines

The cloned cell lines, derived from VM/Dk P497, A 8, B 2, B 6, C 12, D 2 and F 1 were derived as previously described [6]. These cell lines were shown to be free of contamination with Mycoplasma spp by Hoechst 33258 staining using a methodology previously described [9] and stored in the liquid phase of a liquid nitrogen freezer. The methods used for the routine maintenance of cell cultures have previously been described [6].

Characterisation of the clones

Details of the in-vitro genotypic and phenotypic characteristics of the clones are given in [6] and the tumourigenicity studies as well as the morphological, histopathological and immunocytochemical characteristics of the tumours which arose from these cell lines are fully described in [10].