

## Brain tumour migration and invasion: The role of *in vitro* model systems

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*Human primary (intrinsic) brain tumours rarely metastasise to distant organs, however they do show a marked propensity for diffuse infiltrative invasion of the contiguous, normal brain tissue. This is arguably the most important biological feature of this group of – predominantly glial – neoplasms. Single neoplastic glia may migrate several millimetres, or even centimetres from the major tumour mass and there is increasing evidence that during the migratory phase these cells transiently arrest from the cell cycle therefore rendering them refractory to therapeutic radiation. Moreover, they are protected from the action of the majority of cytotoxic drugs by virtue of their investment within areas of intact blood-brain barrier. These migratory, so-called “guerrilla” cells later return to the division phase, under hitherto unknown microenvironmental cues, to form local recurrences of the primary tumour.*

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The terms “migration” and “invasion” in the context of oncology frequently tend to be used interchangeably however, strictly speaking migration refers to the simple movement of cells within a tissue without causing specific damage to the host tissue while invasion infers the movement of cells with consequent detriment to the normal cellular elements. Ideally, in order to elucidate the underlying mechanisms and patterns of invasion this phenomenon should be studied in *in vivo* model systems.

Animal models of brain tumour, however, do not generally fulfil the criteria required for such studies. Indeed, while intracranial tumours induced by chemical carcinogens such as ethyl nitrosourea (ENU) do invade the brain, they occur at inconsistent locations and at differing latency (which is long) and incidence. Transplantable glial tumours tend to have rapid growth rates, growing by expansion rather than diffuse invasion and showing only

limited dissemination, generally along the vascular basal laminae. To date the only convincing demonstration of invasion in an animal model comes from the direct xenotransplantation of human biopsy tissue into immunodeficient rats. This model, however, suffers from lack of reproducibility and therefore ineffective statistical accountability.

In order that invasion of glial tumours can be studied in the laboratory it has been necessary to develop a variety of *in vitro* approaches. Although each of these approaches suffers from intrinsic flaws they have collectively provided considerable information regarding the cellular mechanisms and molecular pathways which underlie the process of brain tumour invasiveness.

The design of such models is of great importance since the culture microsystem exerts an influence on the invasive and motile properties of neoplastic cells.

Although a simple scratch across a confluent monolayer facilitates monitoring of migration, more sophisticated techniques have been developed. For example, cells may be seeded into cloning rings on extracellular matrix protein coated substrates and incubated in culture for a 12 hour period, then the ring may be removed and migration away from the cell colony assessed by sequential photomicrography.

It is also possible to examine the movement of tumour cells across the substrate of a culture dish by time-lapse video photomicroscopy. Here, the substrate can be varied and putative chemorepellant or chemoattractant factors, as well as agents that may promote or retard invasion, may be added to the growth medium and their effects studied over a period of many hours. Using this approach the trajectories of cell movement may be tracked and the intervals between cell divisions logged.

Another method employs modified Boyden chambers. In essence, commercially available "Transwell" units, incorporating inserts with polycarbonate membrane filters (porosities of 8 to 12 microns are generally suitable for the study of human neoplastic glia), are set up with a chemoattractant containing medium (eg platelet-derived growth factor or tumour cell conditioned medium) in the lower chamber and cells for assay are seeded onto the top of the filter in the upper chamber (the two chambers are separated by the filter alone). After a pre-determined period of incubation, filters are removed and cells which have migrated to the lower side of the filter may be stained with simple haematological stains such as modified Papanicolaou (Diff-Quik) or by immunocytochemical methods: electron microscopy may also be carried out. Cells on the non-migratory (upper) side of the filter are either ignored, as unfocussed cells, or may be removed by scraping, counts can then be made of migratory cell populations. By coating the polycarbonate filter with a thin layer

of extracellular matrix (ECM) components such as "Matrigel" or with growth inhibited, viable non-neoplastic cells, the migration assay can be converted to an assay for invasiveness. Such assays not only monitor the motile propensity of cells but also require that the cells adhere to and subsequently degrade the ECM in order to permit invasion.

Three-dimensional confrontational models where normal tissue is maintained in the presence of neoplastic glial cells has enabled assessment of invasive potential and has helped to elucidate the interaction between normal and neoplastic cells during invasion. In early studies, cell lines derived from ENU-induced brain tumours in the rat were confronted with embryonic chick heart fragments and this system has proved to be of value in assessing invasion of human brain tumours. Indeed, the degree of invasiveness in this system has been shown to correlate well with malignancy and clinical evolution of the neoplasms. For example, malignant gliomas invade into the normal tissue and destroy it while meningiomas surround but do not invade the "host" tissue. In other three-dimensional systems re-aggregated foetal brain has formed the target for *in vitro* invasion by both human and experimental animal brain tumour cell lines or short-term, early passage cultures maintained as either monolayer cultures or as multicellular tumour spheroids. Optic nerve, too, has provided a neural target with which to study invasion and has yielded information concerning the phagocytic activity of human and animal gliomas during invasion, as well as providing morphological evidence of interaction with the ECM.