

MODULATION OF CELLULAR MIGRATION IN A MOUSE MODEL OF NERVOUS SYSTEM TUMOUR IN TWO AND THREE DIMENSIONS

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Astrocytomas are the leading form of brain cancer in humans. Reilly et al developed a mouse model of Neurofibromatosis I involving the heterozygous mutation of two tumour suppressor genes, Nf1 and p53. These mice develop a variety of tumour types including numerous astrocytomas, ranging from low-grade to highly infiltrative glioblastoma multiforme. Tumours from these mice were resected and partitioned to allow for culturing and simultaneous histological examination of differing grades of tumour cells (Reilly, unpublished observations). The primary goal of this study is to create a milieu that favors cellular migration as well as an in vitro model allowing modulation of migratory potential. To perform the scratch wound model, cells were grown to 80-100% confluency prior to withdrawal of serum for 8 hours. A cellular response to serum deprivation is that of increased expression of epidermal growth factor (EGF) receptor. By 8 hours, addition of 12ng/ml EGF was sufficient to promote migration of cells into the wound produced by scratching cells from the culture vessel using a small bore plastic pipette tip. The cells were then examined intermittently at various locations for 18-36 hrs. The numbers of cells migrating into the wound are presented along with the rates. Factors known to affect cellular migration such as MAP kinase and AKT were manipulated in order to determine effects on migration across stages of tumour development. We show a mean migration of approx. 820 tumour cells into the wound area of serum deprived, EGF treated cells. MAP kinase inhibition led to a 65% reduction in migration while AKT inhibition led to greater than 80% attenuation. Accompanied by the low migratory potential of the cells was a dramatic increase in apoptosis. Similarly, mouse tumour cells as well as normal astrocytes were grown on, as well as inside artificial extracellular matrices. Tumour cells were shown to interact with the matrix during migration by digesting fluorescently labeled collagen while normal astrocytes, although they migrated similarly, did not have such interaction with surrounding collagen indicating a role for matrix metalloproteases in tumour cell migration. This three dimensional representation of tumour cells may reflect a translational model, between the two dimensional cell cultures and the live animal. These models provide an opportunity to directly examine migratory responses of tumour cells in two and three to better understand the infiltrative nature of many tumours of neural tissue origin.

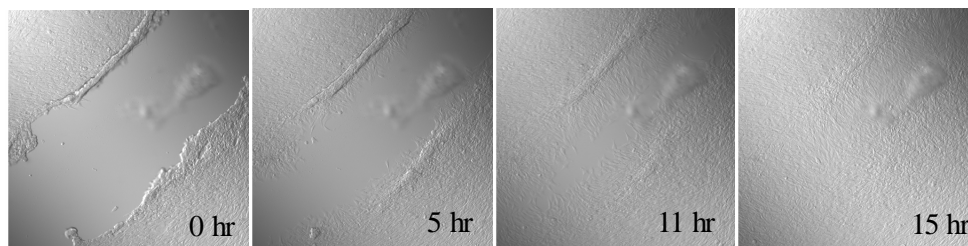


Figure 1. Migration of KR158 mouse astrocytic tumour cells at various timepoints.