Poliovirus Receptor CD155 Over-Expression Effect on Migration in C6 Glioma Cells

Zahra Bhaiwala[1,*], Christine Marshall[2]

[1]Columbia College, Columbia University, New York, NY 10027, [2]Phillips Academy, Andover, MA, 01810

Abstract

Cell migration plays a key role in brain cancer invasion, an early step in metastasis, and proteins that regulate migration are often up-regulated in tumor cells. The poliovirus receptor CD155 has recently been shown to affect migration levels of select malignant glioma strains, fueling the exploration of treating brain cancer with oncolytic virus recombinants. In the beginning phase of experimentation, we analyzed the migratory behavior of control C6 gliomas through two rounds of transfilter assays. In order to explore the function of CD155 in glioma migration, we then conducted two over-expression experiments with the use of a full-length cDNA expression vector and compared the resulting migration rates to control data. We found that an average of 304.25 control cells cross our transfilters after 5.5 hours, and an average of 354.5 cells transfected with the PVR expression vector. Our results reflected a 16.5% increase in cell migration due to an increased presence of the protein CD155. In order to confirm that CD155 was expressed in the rat C6s, we subsequently conducted two Western Blots: one comparing control C6s and transfected C6s, and the other running control C6s next to a human U87 glioma control cell line. The blots establish that CD155 is indeed present in both of these human and rat strains of glioma, and that our over-expression was successful.

Key words: Glioblastoma multiforme; tumor migration; poliovirus receptor CD155; protein over-expression.

Introduction

After the human brain completes its development soon after birth, the vast majority of its cells enter the G0 phase, in which they never divide again. One exception to this rule is when a brain tumor develops as a result of abnormal, unregulated growth of cells. The atypical brain cells re-enter the cell cycle because of alterations in any of a large number of genes that control cell division and growth. Astrocytomas and oligodendrogliomas are types of gliomas that are the most common primary tumors of the adult brain. Primary brain tumors arise from cells of the brain itself rather than traveling, or metastasizing, to the brain from another location in the body like other known forms of cancer. Tumors are generally classified in four grades, with grade 1 being the most benign and grade 4 being the most malignant. Signs that the tumor is growing rapidly include cells undergoing division (mitosis), the presence of newly-formed blood vessels (angiogenesis), and evidence that the tumor is outgrowing its blood supply (necrosis). Typically, malignant gliomas show an area of central necrosis surrounded by a highly cellular rim of viable tumor. Malignant gliomas have remained resistant to therapy and are capable of spreading long distances within the brain (Madsen, 2006).

In glioblastoma multiforme (GBM), the most aggressive form of brain cancer, tumor cells disperse so extensively that common treatment approaches such as surgical resection or radiation therapy are not effective in checking progression. Unfortunately, significant infiltration of normal brain matter is not limited to the most malignant of tumors. The invasive behavior expressed by all levels of malignant gliomas has limited the effectiveness of local therapies and contributes to the high mortality rate seen in these tumors. The position of the tumor is also crucial. Brain stem gliomas are particularly difficult to treat, regardless of their grade. The brain stem is such a complicated and delicate part of the brain that completely removing the tumor is highly unlikely. Unfortunately, high doses of radiation are not recommended for treatment, as this may cause too much damage to the normal brain stem (Giese, 2003).

Having an agent that blocks migration is key to managing glioblastoma multiforme. Interventions to control the spreading of glioblastoma multiforme have the potential to slow the clinical course of the disease and improve overall survival rates. Preventing glioma invasion has the potential to convert this highly malignant tumor into a focal disease, which could then be effectively treated with focal therapies, such as radiation and surgery (Madsen, 2003; Gillespie, 1999).

Received Jan. 2, 2011. Accepted Jan. 26, 2011. Published April 1, 2011.
*To whom correspondence should be addressed:
Columbia College, Columbia University, New York, NY 10027.
zsb2104@columbia.edu

Invasion and Migration of Malignant Gliomas

Tissue invasion by malignant gliomas is a multi-step process. Central to this is the ability of transformed cells to crawl through the extracellular matrix. The initial step requires receptor-mediated adhesion of tumor cells to matrix proteins, followed by a second phase of degradation of the matrix by tumor-secreted proteases. The ability of invasive glioma cells to navigate these diverse anatomic structures and molecular substrates raises the question of whether specific mechanisms and phenotypes of invasive cells are involved in dispersion following specific pathways (Gillespie, 1999).

Gliomas typically invade the brain by migrating long distances through white matter tracts and by infiltrating cortex and subcortical gray matter structures. When migrating through brain white matter, gliomas move in a manner remarkably similar to that previously described for neural progenitor cells. Given the submicrometer size of the extracellular spaces that are present in the tightly packed neuropil of the brain, most other motile cells would likely find it impossible to migrate because there would be no room to allow a lamellipodium to form. The unique form of motility shared by gliomas and neural progenitors reflects adaptations that these cells have to make to a uniquely challenging environment with small effective pore sizes (Beadle, 2008). One of these adaptations is in how glioma cells use myosin II, the major source of cytoplasmic contractile force (Gillespie, 1999). Myosin II is absolutely required for migration in the brain, where its major role is to push the bulky nucleus and cell body through the small pores found within the brain matrix. This feature distinguishes glial cells from other cancers because other cancers do not require myosin II for the collapse of their nucleus. The progenitor-like motility of glioma cells represents a method of migration that is specifically adapted for traversing the densely packed neuropil of the mature brain (Beadle, 2008).

Poliovirus

Poliovirus, among other human pathogenic viruses, is being studied increasingly along with the development of novel therapeutic agents against malignant glioma. Poliovirus, the causative agent of poliomyelitis, is classified within the enterovirus genus of the family Picornaviridae. Poliovirus is composed of a RNA genome and a protein capsid. The genome is single-stranded positive-sense RNA genome that is about 7500 nucleotides long, while the viral particle is about 30 nanometers in diameter with icosahedral symmetry. Because of its short genome and its simple composition, poliovirus is widely regarded as the simplest significant virus. First isolated in 1909 by Karl Landsteiner and Erwin Popper, poliovirus has become one of the most well-characterized viruses, and has become a useful model system for understanding the biology of RNA viruses.

There are three serotypes of poliovirus, PV1, PV2, and PV3, each with a slightly different capsid protein. The capsid proteins define cellular receptor specificity and virus antigenicity. PV1 is the most common form encountered in nature, however all three forms are extremely infectious. Wild polioviruses can be found in approximately 10 countries. PV1 is highly localized to regions in India, Pakistan, Afghanistan, and Egypt, but following outbreaks of poliomyelitis in 2003–2004 it remains widespread in West and Central Africa. Wild poliovirus type 2 has probably been eradicated; it was last detected in October 1999 in Uttar Pradesh, India. Wild PV3 is found in parts of only five countries (Nigeria, Niger, Pakistan, India, and Sudan) (Merrill, 2004).

Poliovirus, like other picornaviruses, is characterized by a single positive strand genomic RNA. Infection occurs via the fecal-oral route; meaning that one ingests the virus, which then replicates within the alimentary tract. In 95% of cases only a primary, transient presence of the virus in the bloodstream occurs (called a viremia) and the poliovirus infection is asymptomatic. In about 5% of cases, the virus spreads, and replicates in other sites such as brown fat, the reticuloendothelial tissues, and muscle. This sustained replication causes a secondary viremia, and leads to the development of minor symptoms such as fever, headache and sore throat. Paralytic poliomyelitis occurs in less than 1% of poliovirus infections. Paralytic disease occurs when the virus enters the central nervous system (CNS) and replicates in motor neurons within the spinal cord, brain stem, or motor cortex, resulting in the selective destruction of motor neurons; leading to either temporary or permanent paralysis and, in rare cases, to respiratory arrest and death (Pliaka, 2007). There are two key mechanism by which poliovirus evades the immune system. First, it is capable of surviving the highly acidic conditions of the gastrointestinal tract, allowing the virus to infect the host and spread throughout the body via the lymphatic system. Second, because it can replicate very quickly, the virus overwhelms the host organs before an immune response can be mounted. In addition, there are more than two such mechanisms; additional ones include inhibition of RNA and protein synthesis, and inhibition of protein secretion, both of which interfere with the IFN respsonse. Fully assembled poliovirus leaves the confines

of its host cell 4 to 6 hours following initiation of infection in cultured mammalian cells. The mechanism of viral release from the cell is unclear, but each dying cell can release up to 10,000 polio virions (Merrill, 2004).

CD155/PVR

The cellular life cycle of poliovirus is initiated by binding to the cell surface receptor CD155. The virion is taken up via endocytosis, and the viral RNA is released. Translation of the viral RNA occurs by an IRES-mediated mechanism. The polyprotein is then cleaved, yielding mature viral proteins. The positive-sense RNA serves as template for complementary negative-strand synthesis, producing double-stranded replicative form (RF) RNA. Many positive strand RNA copies are produced from the single negative strand. The newly synthesized positive-sense RNA molecules can serve as templates for translation of more viral proteins, or can be enclosed in a capsid to form progeny virions. Lysis of the infected cell results in release of infectious progeny virions.

The presence of CD155 is thought to define the animals that can be infected by poliovirus. CD155 has only been thought to be on the cells of humans, higher primates, and Old World monkeys. Poliovirus is however strictly a human pathogen, and does not naturally infect any other species. CD155 is a Type I transmembrane glycoprotein in the immunoglobulin superfamily. Commonly known as Poliovirus Receptor (PVR) due to its involvement in the cellular poliovirus infection in primates, CD155's normal cellular function is in the establishment of intercellular adherens junctions between epithelial cells. Like many other receptor molecules used by picornaviruses, CD155 is a long, highly glycosylated, single-span surface molecule. While its predominant function is related to cellular adhesion and activation, CD155 also activates natural killer cells and has been reported to play a role in cell motility and tumor cell invasion. It consists of 3 successive Ig-like domains (D1, D2, and D3), a transmembrane domain and a C-terminal cytoplasmic domain. The human CD155 gene is expressed in 4 splice variants (α, β, γ, and δ) of which 2 variants (β and γ) are lacking the transmembrane domain and are released from the cell subsequent to their expression. The CD155α and CD155δ isotypes differ only in their cytoplasmic domains and can both function as PV receptors Zhang, 2008).

CD155 protein binds specifically to the extracellular matrix component vitronectin. Confirming the activity pattern of the CD155 promoter in the developing spinal cord, morphogenic factors active in the floor plate and notochord—the transcription factors sonic hedgehog (shh) and its downstream gli effectors—strongly activate the CD155 promoter and induce CD155 expression. Both shh and gli transcription factors have been implicated in the oncogenesis of neuroectodermal tumors. Thus, the role of shh and gli transcription factors in CD155 gene regulation suggested that CD155 expression may occur ectopically in neuroectodermal malignancies. Evidence for CD155 expression in neuroectodermal tumors stems mainly from studies of neuroectodermal tumor cell lines that are susceptible to oncolytic poliovirus-based agents (Solecki, 2002).

Oncolytic Applications

Viral oncolysis has been recently recognized as a new development in the treatment of malignant glioma. Oncolytic viruses must specifically target tumor cells, a property related to the fact that tumor cells often have aberrant innate immune reponses. The first reported incidences of viral oncolysis, over a century ago, were due to nonintended exposure to naturally occurring viruses or after administration of live attenuated vaccine strains. In the last twenty years, new prospects for genetically manipulating viruses have opened possibilities for increasing the tumor specificity and lowering the toxicity of oncolytic viral agents. These efforts have given rise to oncolytic adenoviruses, herpesviruses, reoviruses, vesicular stomatitis virus, and most recently, polioviruses. The antineoplastic effects of oncolytic viruses are subject to multifaceted interactions with the host cell. The primary prerequisite for viral oncolysis is expression of cellular receptors mediating viral entry in malignant cell types. In preparation for clinical applications using oncolytic viruses, analysis of viral receptor expression in the target tissue is highly desirable in order to select tumor types and patients most likely to respond favorably to therapeutic intervention.

Cell adhesion molecules of the immunoglobulin superfamily are aberrantly expressed in malignant glioma. Amongst these, the human poliovirus receptor CD155 provides a molecular target for therapeutic intervention with oncolytic poliovirus recombinants. Analysis of 6 cases has indicated that CD155 over-expression is commonly associated with high-grade malignant glioma (HGL). CD155 expression levels in tumor tissues corresponded to those in primary tissue cultures derived from the tumors. Furthermore, CD155 expression in primary glioma explant cultures was equivalent to that found in established glioma cell lines used in preclinical evaluations of oncolytic poliovirus recombinants. Poliovirus has been genetically modified through insertion of regulatory sequences derived from human rhinovirus type 2 to selectively replicate within and destroy cancerous cells. Successful oncolysis with the use of poliovirus infection depends directly on the presence of CD155 in targeted tumors (Sloan, 2005).

Poliovirus has a plus-strand RNA genome, the translation of which depends on a tissue-specific internal ribosomal entry site (IRES) within the 5' untranslated region of the viral genome, which is active in cells of neuronal origin and allows translation of the viral genome without a 5' cap. Non-transductional targeting involves altering the genome of the virus so it can only replicate in cancer cells. This can be done by either transcription targeting, where genes essential for viral replication are placed under the control of a tumor-specific promoter, or by attenuation, which involves introducing deletions into the viral genome that eliminate functions that are dispensable in cancer cells, but not in normal cells. Gromeier et al. replaced the normal poliovirus IRES with a rhinovirus IRES, altering tissue specificity (Gromeier, 2000). The resulting PV1(RIPO) virus was able to selectively destroy malignant glioma cells, while leaving normal neuronal cells untouched.

Although the poliovirus itself has been demonstrated in previous studies to have an oncolytic effect in certain types of gliomas, it is still somewhat unclear what the actual role of the receptor CD155 might have, if any, in this relatively new discovery of cancer treatment through viral infection. In glioblastoma, the most aggressive form of brain cancer, tumor cells disperse so extensively that current treatment approaches such as surgical resection or radiation therapy have little effect in checking progression. The survival of patients with malignant gliomas, and a median survival time of patients with malignant gliomas is still anticipated as a bleak 12 to 14 months. Invasive cells remaining after the surgical resection significantly contribute to the demise of the patient. Any successful treatment will have to treat the invasive portion of the tumor and the core lesion. Specifically targeting invasive glioma cells remains an interesting concept because invasiveness must be a common characteristic of most tumor cells remaining after surgical resection. Because it has been increasingly shown that glioma invasion is regulated by distinct trigger mechanisms, downstream effector molecules of the invasion process represent the best treatment targets. It has been previously found that CD155/PVR was highly expressed in both U87 human glioma cells and primary glioblastoma tumor tissue, and that inactivation of CD155/PVR reduced cell migration in vitro (Sloan, 2005). These findings suggest a novel role for CD155/PVR in regulating motility and has prompted me to explore this function further in the C6 rat glioma cells we have here in lab by conducting an over-expression experiment to mirror the relationship that has been found in the knockdown experiments.

Materials and Methods

Cell Culture

C6 rat glioma cell lines (Canoll Lab, Columbia University) were cultured in Dulbecco's Modification of Eagle's Medium (DMEM) with 4.5 g/L glucose, L-glutamine, and sodium pyruvate supplemented with 10% Fetal Bovine Serum and 1X Penn Strep (Mediatech). Cells were incubated at 37°C in a NAPCO Series 8000 WJ CO2 incubator under a humidified 5% CO2 atmosphere. Cells were passaged every seven days onto fresh poly-L-lysine-coated T75 flasks, and medium was changed every three days.

Transfection of cDNA expression vector

We obtained glycerol stocks of E. coli transformed with a 4.395 kb pCMV-SPORT6 expression vector (Image ID: 3902226, American Type Culture Collection), which used a 50 μg/μl ampicillin marker and was stored indefinitely at -80°C. DNA was isolated according to standard plasmid preparation procedures and absorbance was measured at 260 nm via spectrophotometer (BioMate).

One day prior to transfection, the cells were trypsinized from the T75 flask and added to 10 mL of growth medium. The mixture was then spun in a tabletop centrifuge (2000rpm for 5 minutes) and resuspended in 5 mL of antibiotic-free growth medium. Cells were triturated and then counted by inserting 10 μl into a hemocytometer and approximately 5x105 cells were plated into 60 mM tissue culture dishes containing 3 ml of growth medium.

In two separate sterile eppendorf tubes, the appropriate amount of DNA (based on absorbance value) and 20 μl of Lipofectamine 2000 reagent were diluted in 50 μl of serum-free medium. Both tubes were mixed gently and incubated for 5 minutes at room temperature. The tubes were combined, incubated for another 20 minutes and the entire mixture was added to the transfection dishes. 6 hours after transfection, the media was changed to regular growth media (DMEM + 10% FBS + P/S). Cells were allowed to grow for 48 hours prior to transfilter assays.

Transfilter Migration Assay

In preparation for the migration assay, the C6 cells were trypsinized from the tissue culture dishes, added to 5 mL growth medium, and spun at 1600 rpm for 5 minutes. Cells were resuspended in 1-2 mL of serum-free medium and kept on ice. 3μm pore Fluoroblok transfilters (BD Biosciences) were coated with 10% type 1 rat tail collagen (BD Biosciences) in sterile de-ionized water at 37°C for 1 hour. 500 μl of serum-containing growth medium was added to the bottom of each well beneath the filter to stimulate migration in a single direction (top to bottom). Cells were counted and the number was adjusted so that 5x105 cells were added to the top of each transfilter in a 200 μl mixture. Cells were left in the incubator to run on the filters for 5.5 hours.

Post-migration, cells were fixed in 4% paraformalde-

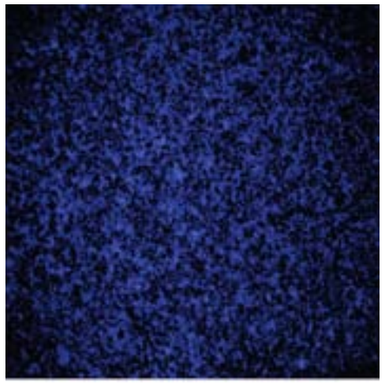


Figure 1 *C6 Control cells remaining on top of the filter after a 5.5 hour migration period.*

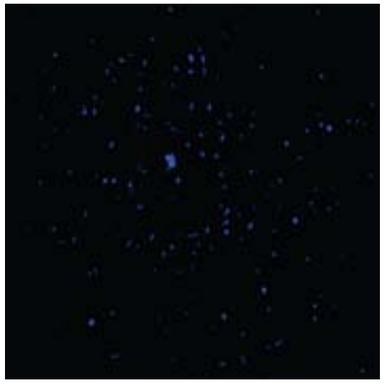


Figure 2 *C6 Control cells at the bottom of filter after 5.5 hour migration period.*

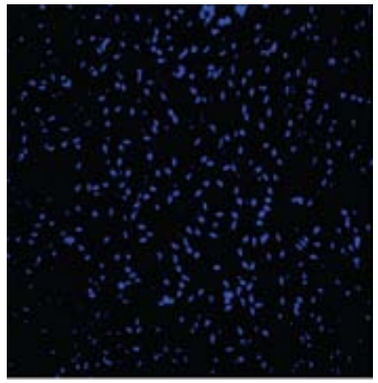


Figure 3 *PVR-Transfected cells at the bottom of filter after 5.5 hour migration period.*

hyde at 4°C for 20 minutes. After 3 washes with phosphate-buffered saline (PBS) at room temperature, the cells were stained with the nuclei stain hoechst (Calbiochem/EMD) at a 1000X dilution of 10µg/10µl for 30 minutes at 4°C. The cells were washed again 3 times with PBS and then plated onto glass coverslips for analysis.

Data Analysis

The number of nuclei on the bottom of each of both control filters was counted in two low power fields (25X) of a Zeiss Axioplan microscope and averaged. The nuclei on the top of the filters was also counted in two low power fields and averaged in order to further assess the rate of migration in normal C6 cell lines.

Lysates

Lysis Buffer (RIPA buffer) was prepared (1 M ph 8 Tris, 5 M NaCl, 20% NP40, 10% SDS, distilled H20 up to 50 mL) and stored at 4°C. 5X Running Buffer (7.5 g Tris, 47 g Glycine, 25 mL 10% SDS, distilled H20 up to 500 mL), 5X Transfer Buffer (9.5 g Tris, 47 g Glycine, distilled H20 up to 500 mL), and 10X TBS (6 g Tris, 44 g NaCl, dH20 up to 500 mL, pH 7.4) were prepared and kept at room temperature.

0.5 mL of EDTA was added to 4.5 mL of lysis buffer to make a 10X stock of protease inhibitor cocktail. The cocktail was then diluted 1:10 in lysis buffer (50µl to 450µl). Cells were washed twice with PBS and 250µl of the lysis buffer mixture was added directly to the tissue culture dish. Cells were scraped and pipeted to an epindorph tube, vortexed for 10 seconds and returned to ice, and then vortexed for 30 seconds. Cells were then spun for ten minutes at 1600 rpm and the supernatant was pulled off into a new epindorph. The lysates were snap-frozen with liquid nitrogen and stored at -80°C until quanitation.

Western Blot Analysis

CD155 expression levels were determined by using a Western blot procedure. 24 µl protein lysate was mixed with 8 µl 4X sample buffer (20 mL 40% Glycerol, 4 g SDS, .01 g .02% Bromophenol-blue, 5 mL beta-mercaptoethanol (BME), 45mL dH20) and boiled for ten minutes. 10% stacking and separating acrylamide gels were prepared according to PAGE Recipe Calculator (Chang Bioscience) and placed into the chamber (VWR), followed by the addition of 1X running buffer up to the brim of the apparatus. The protein mixture, along with Prestained Standard (BioRad) was then loaded into the wells and run at 110V for approximately two hours. After the gel was run, it was placed directly into 1X transfer buffer. The nitrocellulose was prewet in sterile water to activate and the gels were subjected to overnight electrophoretic transfer (.03amp) following standard procedures. Membranes were rinsed in sterile water and then blocked with 50 mL of Tris-buffered saline Tween-20 with 0.25 g BSA (TBSTB) + 5% milk for one hour on the shaker at room temperature. After soaking, the blots were treated with Anti-Necl-5 rat monoclonal antibody Clone 1A8-8 (Gift of Dr. Yoshimi Takai, stored at -80°C) diluted 1:1000 in TBSTB and incubated overnight at 4°C. After the removal of the primary antibody and 3 consecutive ten-minute washes of TBST, the last with TBSTB + 5% milk, the membranes were treated with secondary Anti-Mouse IgG-Alkaline phosphatase antibody produced in goat (SIGMA, stored at 4°C) diluted 1:10,000 in TBSTB and set on the shaker for 1 hour. After three thorough rinses of the blots with TBST, they were treated with BM Purple (Roche) until the appearance of protein bands—after which they were stored at 4°C in TBST.

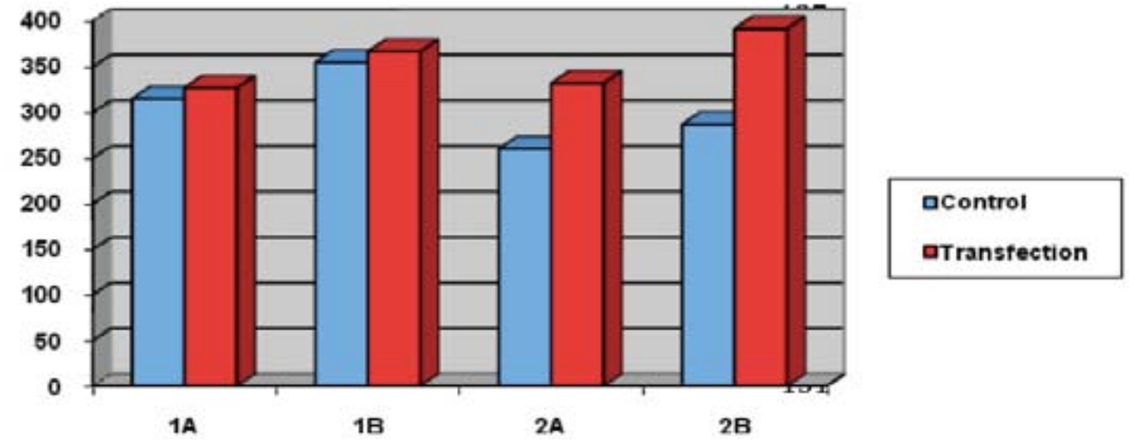


Figure 4 *Migrating C6 cells: Control vs Transfection with PVR Expression Vector*

Results

With two successful Western Blots **(See Figures 5 and 6)**, we were able to formulate a comprehensive picture of C6 glioma migration influenced by transfection of PVR. To determine if increased synthesis of CD155 affects the migration of C6 glioma cells, we captured two images of each control and transfection well, as well as one field each of the cells remaining on the top of the filter **(See Figures 1, 2, 3)**. It was not immediately prevalent through qualitatively assessing cell numbers that a substantial increase in cell migration occurred between the control and transfected cells, but a detailed count revealed an increase.

The nuclei counts for the control fields were 315, 355, 260, and 287, respectively. The numbers of cells transfected with the PVR expression vector that transversed the matrix were 327, 367, 332, and 392. The average of migrating control cells was 304.25, in comparison with the transfection average of 354.5. Our data reflect an average percent increase of 16.5%. By conventional standards according to a statistical T-test (two-tailed P-value of 0.0957), this difference is not considered to be remarkably significant. It is important to note, however, that the second round of transfilter assays yielded a 32.4% increase in glioma migration as a result of the transfection **(See Figure 4)**.

In the first Western Blot, which compared CD155 protein levels in C6 control cells to C6 cells transfected with the PVR over-expression vector, we could confirm from the location of the bands (around 40 kDA, just like the mass of CD155) that the antibody did recognize the

Image Content	DNA Absorbance (µg/ul)	Nuclei Count	Avg. of Assay 1 Control: 335 Tfect: 347
			Avg Percent Increase: 3.5%
Control 1A		315	
Control 1B		355	Avg of Assay 2 Control: 273.5 Tfect: 362
Control 2A		260	Avg Percent Increase: 32.4%
Control 2B		287	
Tfect 1A	.3	327	Avg of All Control: 304.25 Tfect: 354.5
Tfect 1B	.3	367	Avg Percent Increase: 16.5%
Tfect 2A	.4	332	
Tfect 2B	.4	392	Two-tailed P value = 0.0957

human protein and that it is in fact present in the cells. Because the band on the right, the transfected band, is so much darker, we can also assume that our over-expression last term was successful and that any impact on migration we saw was directly correlated to the up-regulation of CD155 in the C6 cells **(Figure 5)**. In the second Western Blot, which compared C6 and U87 control glioma cells, we can observe two distinct, dark bands (again located around 40 kDA) **(Figure 6)**. This is a strong indication that both C6 and U87 glioma cells naturally contain comparable and substantial levels of CD155 protein.

Discussion

We were attempting to mirror the results of a reputable knockdown study conducted by the Department of Physiology at Tufts University School of Medicine, (Boston, Massachusetts) and the Department of Neurobiology at Yale University School of Medicine (New Haven, Connecticut). This study showed that a knockdown of CD155 by FALI in U87MG cells resulted in a significant (16 to 22%) decrease in trans-well migration (Sloan, 2005). Because the siRNA expression vector was not available to conduct a knockdown experiment, our study would be used to perhaps mirror the relationship that has been found previously in the knockdown of CD155.

We expected that over-expression of CD155 would significantly increase migration based on the expression of protein levels indicated by Western Blot analysis. If the over-expression were successful, we would expect that the knockdown that we were unable to conduct out of lack of a RNAi expression vector would also decrease migration.

Despite our predictions earlier in the year, there did not appear to be a tangible visible difference between the control pictures and the transfected pictures for this experiment as a whole. One may notice that in general the transfected cell fields were clearer, and that the control fields contained a lot of processes (especially in Transfilter Assay 1) **(Figure 3)**. This could be possibly interpreted as the presence of CD155 speeding up the growth of cells. If a knockdown in U87 glioma decreased migration of cells, we could infer that CD155 possibly enhances the tumor and may serve as a catalyst for tumor growth. CD155 does not appear to affect the shape or size of the cells.

Without the Western Blots, which were conducted towards the end of our study, we were not able to detect whether or not the transfection substantially increased the presence of CD155 or by how much. This led us to question whether or not CD155 was actually present in C6 cells. We supposed that pursuing this inquiry would allow us to make further distinction between C6 cells and human strain glioma. At the very least, we would find our results to confirm an expected difference between rat and human models.

We predicted that increased CD155 expression in glioma cells was important for migration of the tumor cells in vivo. While our results indicate promise, only two successful experimental replicates were conducted and the variation observed was large—precluding a sound conclusion on the role of CD155 in migration. An additional control, a transfection with a plasmid encoding another protein, would be needed to add to future experiments in order to determine if increased synthesis of any other protein would have the same effect on migration. We would also hope to obtain the siRNA in order to knock down PVR, which was not available at the time.

The challenge we faced during this experiment was the fact that CD155 is thought to be a uniquely human protein, because poliovirus only naturally infects humans. While mice have been genetically engineered to produce

Figure 5 *Western Blot A- C6 Control vs C6 Transfection with PVR*

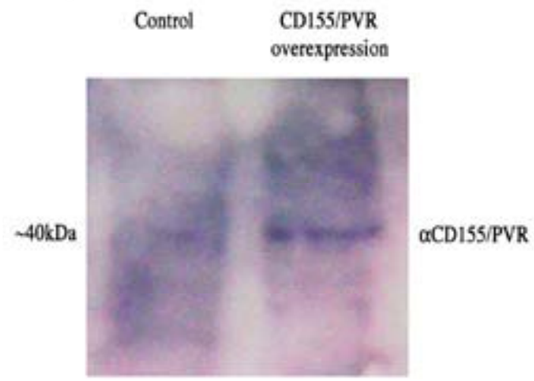
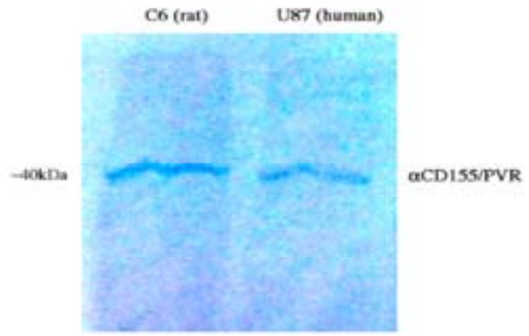


Figure 6 *Western Blot B- C6 Control vs U87 Control*



PVR, we had no predictions as to whether the C6 rat glioma cells would express CD155 at all. CD155 analysis has never been carried out before in C6 gliomas. Furthermore, the primary rat monoclonal antibody we used for our Western Blots was expected to react with Necl-5, the mouse origin of the protein—and not with the human origin CD155. We were essentially testing to see whether our rat gliomas do naturally express the human protein, and if the mouse antibody would recognize the human homolog. Since the blots confirm that CD155 is indeed present in C6 cells, in an amount comparable to that present in U87 human cells, we can confirm that the mouse and human homologs of the protein are similar. Indeed, CD155 and Necl-5 are referred to in combination within scientific dialogue and have virtually the same structure. Yet if the antibody treats CD155 and Necl-5 as the same protein, why is the poliovirus itself specific to humans? Not only do our findings shed light on the strict nature of specificity characteristic to viruses, but these results suggest that CD155 has more than one function besides being the human receptor for poliovirus, and in concurrence with our assay counts, does effect migration on some level. Although some of our counts were not statistically significant, what we do see is that the second assay with a higher concentration of DNA had an overall much larger percentage difference than the control. If we know that the C6 cells contain the human protein and their migration is influenced by it, we can now potentially use rats as a model for CD155/PVR oncolytic treatment in humans.

A study done by the Department of Molecular Genetics and Microbiology at the State University of New York (Stony Brook, NY) demonstrated for the first time that highly attenuated poliovirus recombinants can infect and propagate in cell lines derived from malignant gliomas and, most interestingly, halted tumor progression and eliminated tumors in athymic mice. They proposed that susceptibility of these malignant cells to poliovirus may be mediated by expression of the CD155 gene in glial neoplasms (Gromeier, 2000). As our understanding of the biology of brain cancer progresses, new knowledge about tumorigenesis and tumor biology can be used to diagnose, treat, and prevent this type of cancer. Our experiment may help to support the concept that oncolytic poliovirus recombinants may be the next possible treatment for malignant glioma.

References

Beadle C, Assanah MC, Monzo P, Vallee R, Rosenfeld SS, Canoll P. "The Role of Myosin II in Glioma Invasion of the Brain." MBC: Vol. 19, Issue 8, 3357-3368. August 2008.

Burgoyne AM, Phillips-Mason PJ, Brady-Kalnay SM, et al. "Proteolytic cleavage of protein tyrosine phosphatase mu regulates glioblastoma cell migration." Cancer Research: 6960-6968. August 18, 2009.

Giese A, et al. "Cost of Migration: Invasion of Malignant Gliomas and Implications for Treatment." American Society of Clinical Oncology: 1624-1635. 2003.

Gillespie GY, Soroceanu L, Manning TJ, Gladson CL, Rosenfeld SS. "Glioma Migration can be Blocked by Nontoxic Inhibitors of Myosin II." Cancer Research: 2076-2082. May 1999.

Gromeier M, Lachmann S, Rosenfeld MR, et al. "Intergeneric poliovirus recombinants for the treatment of malignant glioma." Proc Natl Acad Sci USA: 6803–6808. June 6, 2000.

Madsen SJ, Sun CH, Tromberg BJ, Cristini V, De Magalha N, Hirschberg H. "Multicell Tumor Spheroids in Photodynamic Therapy." Lasers in Surgery and Medicine: 555–564. 2006.

Merrill MK, Bernhardt G, Sampson JH, Wikstrand CJ, Bigner DD, Gromeier M. "Poliovirus receptor CD155-targeted oncolysis of glioma." Neuro-oncology: 208-217. 2004.

Pliaka V, Dedepsidis E, Kyriakopoulou Z, Papadopoulou I, Levidiotou S, Markoulatos P. "Use of mutational pattern in 5'-NCR and VP1 regions of polioviruses for molecular diagnosis." Molecular and Cellular Probes: Vol. 21, Issue 4, 267-275. August 2007.

Sloan KE, Stewart JK, Treloar AF, Matthews RT, Jay DG. "CD155/PVR enhances glioma cell dispersal by regulating adhesion signaling and focal adhesion dynamics." Cancer Research: 10930–10937. December 1, 2005.

Solecki DJ, Gromeir M, Mueller S, Bernhardt G, Wimmer E. "Expression of the Human Poliovirus Receptor/CD155 Gene Is Activated by Sonic Hedgehog." The Journal of Biological Chemistry: 277, 25697-25702. April 30, 2002

Zhang P, Mueller S. "Crystal structure of CD155 and electron microscopic studies of its complexes with polioviruses." PNAS: Vol. 105, no. 47, 18284-18289. November 2008.