



Investigation of Targetin, a Microtubule Binding Agent which Regresses the Growth of Pediatric High and Low Grade Gliomas.

Norbert F. Ajeawung, *Laval University*
[Harish C Joshi](#), *Emory University*
Deepak Kamnasaran, *Laval University*

Journal Title: Journal of Pediatric Oncology Nursing

Volume: Volume 1

Publisher: SAGE Publications (UK and US) | 2013, Pages 32-40

Type of Work: Article | Final Publisher PDF

Publisher DOI: 10.14205/2309-3021.2013.01.01.5

Permanent URL: <https://pid.emory.edu/ark:/25593/tf8jr>

Final published version: <http://dx.doi.org/10.14205/2309-3021.2013.01.01.5>

Copyright information:

© 2013 Pharma Professional Services

This is an Open Access work distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License (<http://creativecommons.org/licenses/by-nc/3.0/>).



Accessed December 6, 2022 6:58 PM EST

Investigation of Targetin, a Microtubule Binding Agent which Regresses the Growth of Pediatric High and Low Grade Gliomas

Norbert F. Ajeawung¹, Harish C. Joshi² and Deepak Kamnasaran^{1,*}

¹Department of Pediatrics, Laval University, Québec, Québec, G1V 4G2, Canada

²Department of Cell Biology, Emory University School of Medicine, Atlanta, GA 30322, USA

Abstract: *Background:* Pediatric gliomas, the most common solid childhood neoplasm, manifest unique molecular signatures that distinguish them from adult gliomas. Unfortunately, most studies have focused on adult gliomas and extrapolate the findings to treat pediatric gliomas. In this study, we assessed the efficacy of Targetin, a folate conjugated analogue of Noscapine, on the treatment of pediatric low and high grade gliomas.

Method: An assortment of standard cancer assays were used with different drug doses and experimental durations.

Results: We found that pediatric glioma cells are more susceptible to lower doses of Targetin than parental Noscapine. Targetin functions by disrupting the microtubule network, and can likewise perturb DNA synthesis, delay the cellular transition within the S and G2M cell cycle phases, diminish anchorage independent growth and the migratory/invasiveness of pediatric glioma cells. Moreover, Targetin impairs the expression of several regulators of cancer progression belonging to prominent signalling pathways in pediatric gliomas; including Platelet Derived Growth Factor alpha and some members of the Mitogen Activated Protein Kinase cascade.

Conclusion: Targetin has an excellent anti-neoplastic profile and functions to modulate the expression of several genes belonging to key cancer progression pathways in pediatric gliomas. Collectively, findings from this study highlight the usefulness of Targetin for the treatment of pediatric high and low grade gliomas.

Keywords: Pediatric astrocytomas, drug discovery, molecular therapeutics, microtubules, gene expression, apoptosis.

SUMMARY POINTS

- Targetin is a new microtubule binding agent which is conjugated with a folate ligand (folate group) at the C9 position of Noscapine.
- Targetin is more potent than its parental Noscapine drug.
- Targetin significantly reduces cell viability, proliferation, migration and invasion in a dose and duration dependent manner.
- Targetin significantly inhibits anchorage independent growth in a dose dependent manner.
- Targetin functions by affecting microtubule dynamics leading to the arrest of glioma cells in the S and G2M phases of the cell cycle, and subsequently cell death.
- Targetin also perturbs the expression of several genes involved in cancer progression, and the activity of CDC25A.

- Our evidence collectively suggests that the endowed anti-tumorigenic properties of Targetin (a new microtubule binding drug), makes it appealing for the treatment of pediatric low and high grade astrocytomas, and therefore warrants the need for future clinical trials.

1. INTRODUCTION

Pediatric gliomas comprise a heterogeneous tumour group, representing the most frequent type of central nervous system neoplasms. Like adult gliomas, pediatric gliomas are histologically divided into distinct gradations ranging from groups I to IV as classified by the World Health Organization [1]. The less aggressive, but with the risk of progression to malignancy, are low grade gliomas (WHO grade I and II tumours), and comprise mostly of pilocytic astrocytomas and diffuse fibrillary astrocytomas [2], while the highly lethal forms are high grade gliomas with anaplastic astrocytoma and glioblastoma being the most abundant [3]. Despite these histological similarities, distinct genomic events [4-6] have established unique molecular signatures in pediatric astrocytomas when compared to adult astrocytomas. A physical and functional link was previously established between modulators of these aberrantly expressed pathways and microtubules [7-11]. PDGFRA resides in the primary cilia (a microtubule based organelle) during growth arrest and its

*Address correspondence to this author at the Department of Pediatrics, Laval University, Québec, QC, Canada; Tel: (418) 656-4141; Fax: (418) 654-2753; E-mail: deepak.kamnasaran@crchul.ulaval.ca

downstream targets MEK1/2 are phosphorylated within the cilium and the basal body [7]. Likewise, numerous microtubule disruptors were shown to modulate the function of several MAP kinases [12], further underscoring the potential usefulness of tubulin binding agents in the treatment of diseases like pediatric gliomas.

Unlike current clinically used tubulin binding agents such as Paclitaxel and Vinca alkaloids, that are confounded by complications of toxicity, the opium derived microtubule modulating agent, Noscapine and its analogues, are endowed with enhanced anti-tumour potentials and a safer toxicity profile [13,14]; hence being more appealing for the treatment of childhood neoplasms. Originating from the expression of the folate receptors-alpha FR α receptors on a variety of cancer cell types and using molecular modelling techniques, we recently conjugated a folate ligand (folate group) to the C9 position of Noscapine, to yield a new tubulin binding agent known as Targetin [15]. In this study, we investigated the anti-neoplastic properties of Targetin in a panel of high and low grade pediatric glioma cell lines.

2. METHODS

2.1. Tissue Culture

The pilocytic (R286), anaplastic (UW467) and pediatric glioblastoma (SF188) cell lines used in this study were kindly provided by Drs. Micheal Bibola (Washington University, Seattle, USA) and Chris Jones (Institute for Cancer Research, UK). All cell lines were previously characterized [16-18] and were propagated in Dulbecco's modified eagle medium containing 10% FBS and 1% antibiotic-antimycotic except for the R286, which was grown in the presence of EMEM/F12 supplemented with 10% FBS (Fisher) and 1% antibiotic-antimycotic. The cells were maintained at 37°C and 5% CO₂.

2.2. Determination of the Half Maximal Inhibitory Concentration (IC₅₀)

Targetin was synthesized as previously described [15]. The IC₅₀ of Targetin in pediatric glioma cell lines was determined using the cell titre 96 Aqueous one cell proliferation assay-MTS (Promega). Briefly, 2.0 X 10³ cells were plated in each well of a 96 well plate and allowed to adhere overnight. The cells were treated with varying doses of Targetin and Noscapine, ranging from 0 μ M to 500 μ M for 24 hours [19].

2.3. Assessment of Cell Proliferation and Clonogenicity

The BrdU cell proliferation ELISA assay and anchorage independent growth in soft agarose were undertaken as previously described [19].

2.4. Measurement of Apoptosis

Targetin was administered to 2.0 x 10⁵ cells per well in 6-well plates. Prior to analyses, cells were labelled with annexin V/propidium iodide and subjected to flow cytometry analyses.

2.5. Cell Cycle Analysis

Cells at different stages of the cell cycle were determined as previously described [19]. Briefly, 2.0 X 10⁵ cells were stained with propidium iodide mixed with RNase and subjected to FACS analyses using the BD Biosciences Canto II software.

2.6. Mitochondrial Membrane Permeability Determination

Alteration in mitochondrial membrane permeability was assessed as previously described [20]. Cells were exposed to 40 nM DioC6 (3) and subjected to flow cytometry analyses. The proportion of cells, with compromised and non-compromised mitochondrial membrane, was determined and plotted against respective treatments.

2.7. Assessment of Cell Migration and Invasion

Cell migration and invasion were investigated using the Wound healing and the Matrigel Boyden chamber invasion assays, as previously described [21]. The mean migratory distance and the proportion of invading cells were obtained from five randomly viewed fields with the aid of a microscope and plotted relative to untreated cells that were normalized to a value of 100%.

2.8. Immuno-Fluorescent Cytochemistry

Immuno-fluorescent microscopy was performed as previously described [22]. Briefly 1.0 X 10⁵ cells were cultured on glass slides, fixed with ice-cold methanol, and then incubated with a microtubule binding, primary antibody DMA-1 (Santa Cruz biotech), followed with a rabbit polyclonal protein G-FITC secondary antibody (Abcam). Mitotic bodies were viewed and documented with the Nikon eclipse 80i microscope system.

2.9. PCR Array and Gene Expression Analysis

Total RNAs from R286 and SF188 were used for cDNA synthesis with the Superscript II cDNA synthesis kit as described by manufacturer (Invitrogen). 89 primers for Real-time PCR were designed using Primer Blast (NCBI), commercially synthesized, and then used with the Soofast Evagreen kit (BioRad) and a CFX 96 Real-time PCR machine (BioRad).

2.10. Determination of CDC25A Activity

The cell division cycle 25A (CDC25A) antibody was added to a microtitre plate containing varying concentrations of Targetin (0-100 μ M) and CDC25A assay buffer. The plate was incubated at room temperature for 15 minutes and the activity of CDC25A was measured at an excitation of 482 nm and emission of 520 nm, as described by manufacturer (Cyclex).

2.11. Statistical Analysis

All experiments were performed in triplicate and repeated at least twice. Data were analysed using the

Graphpad prism version 5 software. Multiple comparisons were performed using the ANOVA and post-hoc Bonferonni tests. P-values <0.05 were considered statistically significant.

3. RESULTS

3.1. Targetin Retards Cell Proliferation, Viability and Anchorage Independent Growth

To augment the potency of Noscapiene, we recently conjugated the Folate Receptor Ligand (FR α), folate, with aminonoscapiene to yield a novel tubulin binding compound known as Targetin. Despite similarities in tubulin binding affinities, the IC₅₀ value of Targetin in our panel of Pediatric glioma cell lines (SF188, UW467, R286) were 4 to 11 times lower compared to Noscapiene (Figure 1A). Furthermore, the UW467 anaplastic astrocytoma cell line had a lower IC₅₀ value when compared to the pilocytic astrocytoma cell line (R286) or SF188 glioblastoma cell line; suggesting the histologic grade is an unlikely predictor of sensitivity to Targetin treatments.

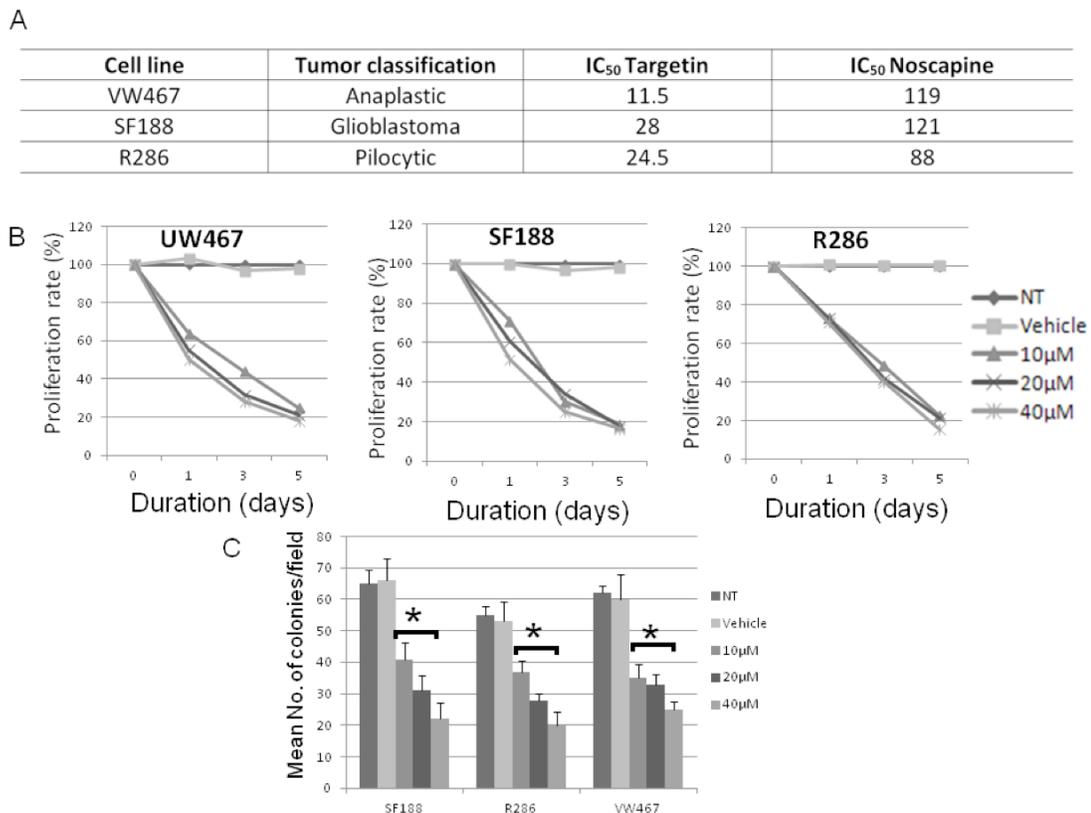


Figure 1: Cytotoxicity profiles of Targetin. (A) Using MTS viability assays, Targetin is demonstrated to be more potent than the parental Noscapiene compound among the panel of 3 pediatric glioma cell lines. (B) Using the BrdU proliferation assay, Targetin dose dependently abrogated the proliferation of all 3 pediatric glioma cell lines over a five day period. NT = untreated. (C) Targetin significantly decreases anchorage independent growth in soft agarose over an 18 day period in a dose dependent manner. NT = untreated. All P-values < 0.05 were considered significant and are represented with an asterisk (*), depicting significant differences between Targetin treated and untreated cells.

These findings prompted us to further investigate the effect of Targetin on cell viability and proliferation during a five day period. BrdU labelling (Figure 1B) and MTS (data not shown) experiments subsequently showed a dose and duration-dependent diminution in cell viability and proliferation in all three cell lines treated with Targetin. Furthermore, the long term assessment of Targetin revealed a significant decrease in anchorage independent growth over an 18 day period, as a consequence of reductions in the size as well as number of colonies that can grow in soft agarose (Figure 1C).

3.2. Targetin Alters Microtubule Networks, and Induces Cell Cycle Arrest, Phosphatidyl Serine Externalization and Dissipation of Mitochondrial Membranes

Using immunofluorescent cytochemical analyses (IFC) of Targetin-treated cells labelled with the anti-Tubulin antibody DM1A, we observed the accumulation of several mitotic cells (Figure 2) which coincided with the arrest of pediatric glioma cells at the G2M phase of the cell cycle (Figure 3A). In addition, we observed populations of cells arrested in interphase consistent

with observed sub-populations of cells arrested or delayed within the S-phase. The observed cell cycle arrest could explain the decline in cell viability and proliferation as seen in treated cells when compared to untreated controls. Furthermore, since the expression of several cell cycle regulators including CDKN1B, CCNA2 and CDC25A (Figure 5C) were altered in glioma cell lines treated with Targetin, we investigated the effect of Targetin on the activity of the CDC25A protein. Even though Targetin could significantly decrease the expression of the CDC25A gene, it failed to abrogate CDC25A protein activity (data not shown); hence suggesting that the mechanism of action for Targetin is limited to disrupting the expression of CDC25A.

Given that we initially observed a dose dependent accumulation of Targetin treated cells within the Sub G0/G1 compartment (Figure 3B): a phenomenon depicting late apoptotic cells, we further assessed early apoptotic cells by virtue of undertaking phosphatidylserine externalization and mitochondrial membrane potential dissipation experiments. We found that Targetin dose dependently and progressively

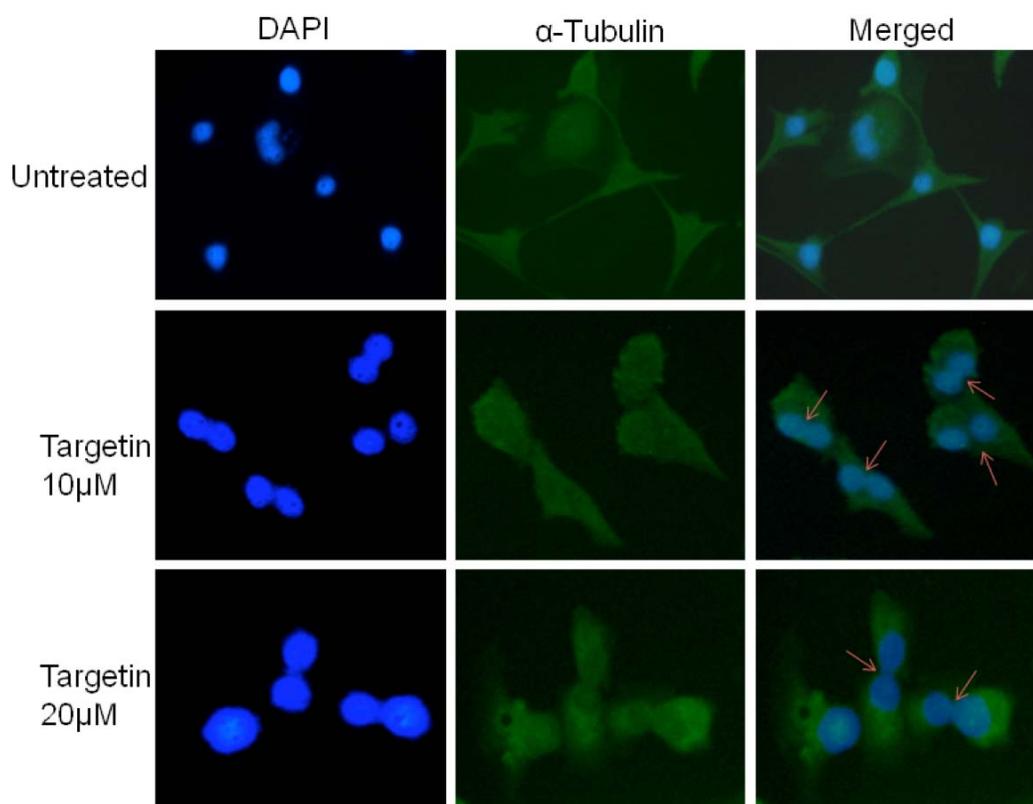


Figure 2: Representative images of Immunofluorescent cytochemistry studies done on the SF188 cell line treated with varying doses of Targetin, and labelled with the DM1A alpha tubulin antibody (Green). Nuclei were stained with DAPI (blue). Notice the appearance of several mitotic cells after 48 hours post-Targetin treatment. Arrows depict cells arrested at mitosis. NT = untreated

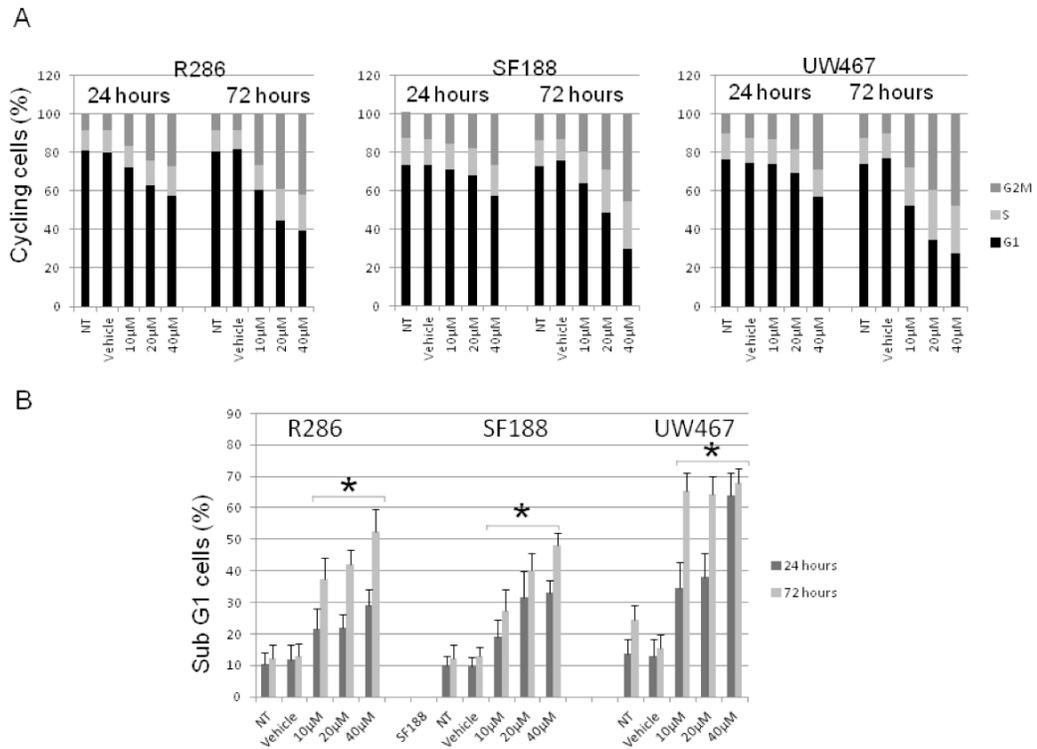


Figure 3: Targetin delays the transition of cells in the S and G2M phases of the cell cycle. **(A)** FAC analyses of 3 pediatric glioma cell lines showing an accumulation of cells within the S and G2M phases, in a dose and time dependent manner, after Targetin administration. **(B)** Flow cytometric quantification of sub-G1 cells in untreated (NT) and Targetin-treated pediatric glioma cells. All P-values < 0.05 were considered significant and are represented with an asterisk (*), which signifies significant differences between Targetin treated and untreated cells.

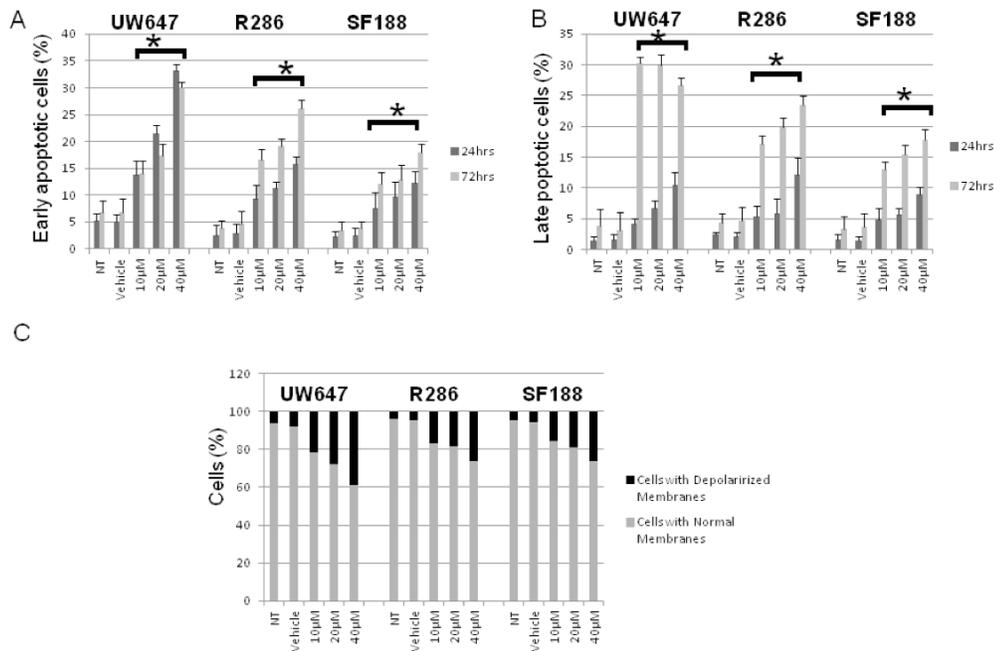


Figure 4: Targetin induces apoptosis. Apoptotic cells were detected by flow cytometry following labelling with either annexin V/Propidium iodide or the DioC6(3) dye. Treatments with varying Targetin doses progressively increased the proportion of **(A)** annexin positive/propidium iodide negative (AV+/PI-) and **(B)** annexin positive/propidium iodide positive (AV+/PI+) cells, depicting the presence of both early and late apoptotic events respectively. **(C)** Assessment of mitochondrial membrane permeability in Targetin-treated and non treated cells labelled with DioC6(3). Targetin dose dependently promoted mitochondrial membrane dissipation. NT = untreated. All P-values < 0.05 were considered significant and are represented with an asterisk (*), depicting significant differences between Targetin treated and untreated cells.

increased both populations of early and late apoptotic cells (Figure 4A, B), as well as promoted dissipation of the mitochondrial membrane potential (Figure 4C).

3.3. Targetin Interferes with Cell Migration and Invasion

Microtubules have a crucial role in regulating cell motility. Analysis of data emerging from our *in vitro* metastasis studies subsequently revealed a dose dependent decrease in the migration and invasion potentials of our panel of pediatric glioma cell lines treated with Targetin when compared to untreated controls (Figure 5A, B). This phenomenon could have originated from decreased microtubule dynamics (Figure 2) and/or changes in the expression of several cytoskeletal/pro-migratory genes including PDGFRA, MMP9 and Vimentin (Figure 5C).

3.4. Targetin Perturbs the Expression of Genes Involved in Cancer Progression

We next questioned whether Targetin could interfere with the expression of genes implicated in cancer progression. Indeed, gene expression profiling of 85 cancer progression genes and 4 housing keeping genes, revealed significant variations in the transcriptional levels between grade I (R286) and grade IV SF188 pediatric glioma cell lines upon having responses to Targetin (Figure 5C). Alterations in gene expression by over two fold were further observed among 29 genes in the R286 pilocytic astrocytoma cell line but in only 14 genes in the SF188 glioblastoma cell line following exposure to Targetin. Remarkably, Targetin significantly decreased the expression of several modulators belonging to pathways aberrantly expressed in pediatric gliomas including PDGFRA,

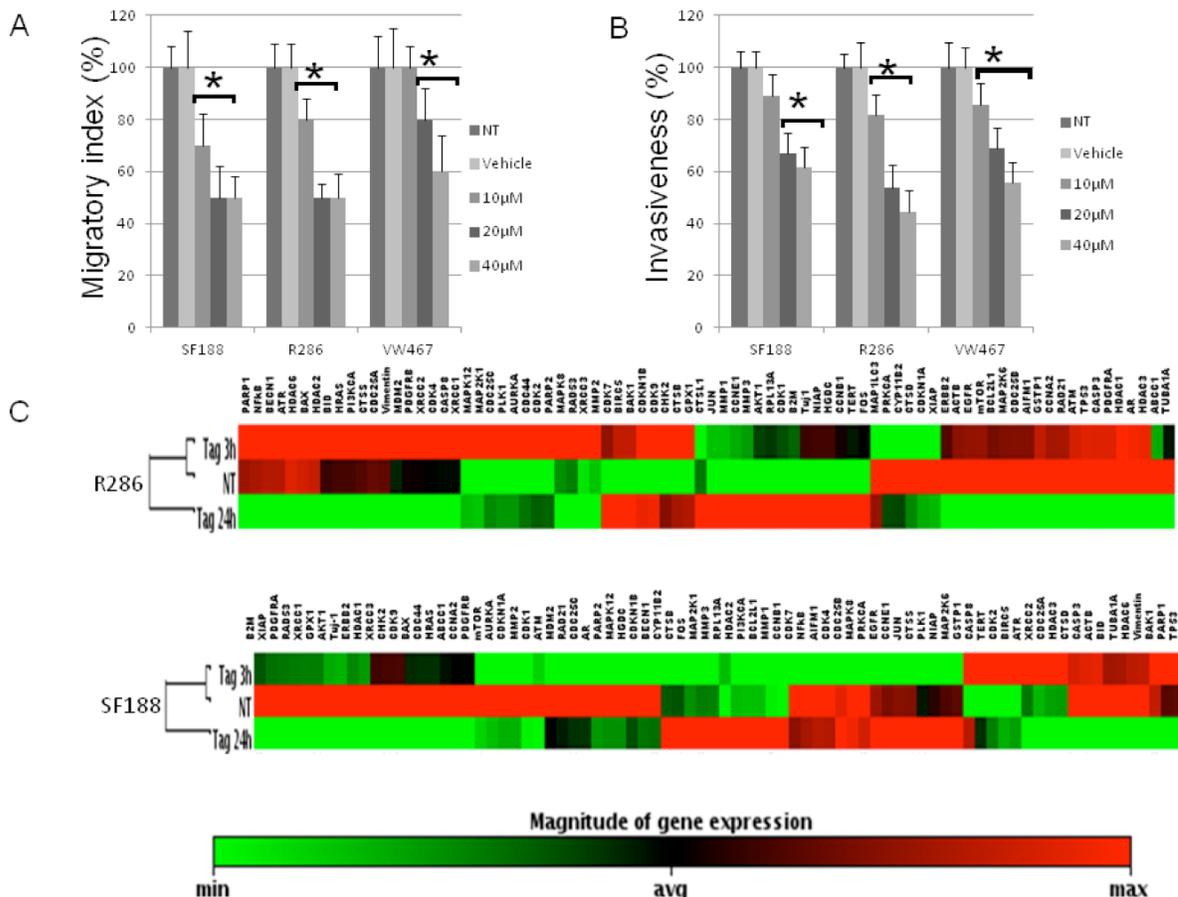


Figure 5: Targetin attenuates cell migration and invasion, and further alters the expression of cancer progression genes. (A) Cell migration and (B) invasion were assessed using the Wound healing and Boyden chamber matrigel assays respectively. Targetin significantly reduced the migratory and invasive potentials of both low and high grade glioma cell lines compared to the negative controls. All P-values < 0.05 were considered significant and are represented with an asterisk (*), depicting significant differences between Targetin treated and untreated cells. (C) Effect of Targetin on the expression of genes involved in cancer progression pathways. Clustergram summarizing the data from Real time PCR array analyses of R286 and SF188, and showing significant changes in the expression of cancer progression genes at 0hr, 3hr and 24hr after treatments with Targetin (Tag). NT = untreated.

MAP2K6, MAPK8, MAPK12, mTOR and HRAS in both the R286 and the SF188 cell lines; suggestive of common mechanistic molecular pathways influenced by Targetin in both high and low grade pediatric gliomas.

4. DISCUSSION

Integrated genomic approaches have delineated unique molecular signatures between pediatric and adult gliomas [4-6], a finding that limits the extrapolation of results from adult clinical studies for the design of similar therapies among children with gliomas. Therefore, specific therapies tailored to pediatric gliomas are anticipated to be more effective. Given their role in a variety of cellular process, microtubules continue to be attractive targets for cancer therapy [23]. Despite their enhanced anti-neoplastic potentials, clinically useful tubulin binding compounds including some belonging to the Vinca and Taxane families are confounded with serious side effects and amenability to acquired drug resistance in cancerous cells [36]. Noscapioids on the contrary, can bind to tubulin without altering the total monomer/polymer mass ratio. In this manner, both in-vitro and in-vivo studies have further concluded that Noscapioids induce only subtle changes in microtubule dynamics leading to the attenuated growth of cancerous cells but with the maintenance of little or no toxicity to non-neoplastic cells [13]. Hence, compounds belonging to the Noscapioid family are anticipated to be favoured for the treatment of a variety of malignancies including pediatric gliomas.

Targetin is a folate conjugated analogue of Noscapiine, that efficiently binds to tubulin, modulate microtubule dynamicity [15] and unlike parental Noscapiine, suppresses the growth of pediatric glioma cells at much reduced doses. Consistent with the pre-clinical mechanistic activity of other Noscapioids [13,14], in pediatric glioma cells, Targetin progressively induced the accumulation of cells in the S and G2M phases of the cell cycle which coincided with decreased DNA synthesis (proliferation) and the appearance of mitotic phenotypes with disrupted microtubule network. Alterations in the microtubule organisation ultimately leads to growth arrest and apoptosis [24] which in the case of Targetin, was associated with the increased externalizations of phosphatidyl serine and mitochondrial membrane depolarization in pediatric glioma cells. The delay in cellular transition within the DNA replication and mitotic phases coupled with Targetin-mediated induction of

apoptosis, profoundly impaired DNA synthesis, cell viability, cell proliferation and anchorage independent growth of both low and high grade pediatric glioma cell lines.

Targetin also significantly reduced the migratory and invasive potentials of pediatric gliomas. A decrease in cell motility and invasion can be triggered by a disruption in the tubulin cytoskeleton which in a normal state orchestrates several facets of cell motility [25]. Alternatively, the decreased expression of pro-migratory genes including PDGFRA, as seen in both the pilocytic and glioblastoma cell line treated with Targetin, could influence the overall migratory/invasive properties of these cells. Prior evidence also indicated that an attenuation of microtubule dynamics is regulated *via* the MAPK signalling cascade [12]. Indeed, we identified Targetin diminishes the expression of several downstream targets like MAP2K6, MAPK8, MAPK12 and mTOR of the MAPK pathway in our panel of pediatric glioma cell lines. Since focal amplification of PDGFRA and the constitutive activation of the MAPK pathway are the most predominant aberrations among both pediatric low and high grade gliomas [4-6], inhibition of the biological signals emerging from these pathways by the microtubule modulating compound Targetin, fulfills promises for improved targeted therapies in the treatment of pediatric gliomas.

5. CONCLUSION

In summary, we have synthesized a new and more potent derivative of Noscapiine, known as Targetin, by conjugating a folate ligand at the C6 position of Noscapiine. Our studies have further overwhelmingly demonstrated high efficacies with the use of Targetin to treat both low and high grade pediatric gliomas, using our panel of glioma cell lines. These precursory findings from this study justify the need for future in-vivo pre-clinical studies in model organisms as well as clinical trials among pediatric patients with gliomas.

ACKNOWLEDGEMENTS

We thank Dr. Kam Kamnasaran for comments on this manuscript; Dr. Manjit Rana for technical assistance; Drs. Michael Bobola (University of Washington, USA), and Chris Jones (Institute for Cancer Research, UK) for providing all cell lines used in this study. This work is supported by a Doctorate Scholarship to NFA from FRSQ, and grants to DK from the CHUQ Foundation, Fondation des étoiles,

Canadian Foundation for Innovation, Laval University Faculty of Medicine Foundation, Natural Sciences and Engineering Research Council, and Fonds de la recherche en santé du Québec, and to HCJ from the National Institutes of Health. DK is a FRSQ scholar.

SOURCES OF FUNDING

This work is supported by a Doctorate Scholarship to NFA from FRSQ, and grants to DK from the CHUQ Foundation, Fondation des étoiles, Canadian Foundation for Innovation, Laval University Faculty of Medicine Foundation, Natural Sciences and Engineering Research Council, and Fonds de la recherche en santé du Québec, and to HCJ from the National Institutes of Health. DK is a FRSQ scholar.

COMPETING INTEREST

The authors have none to declare.

REFERENCES

- [1] Kleihues P, Louis DN, Scheithauer BW, Rorke LB, Reifenberger G, Burger PC, *et al.* The WHO classification of tumors of the nervous system. *J Neuropathol Exp Neurol* 2002; 61: 215-25.
- [2] Karajannis M, Allen JC, Newcomb EW. Treatment of Pediatric Brain Tumors. *J Cell Physiol* 2008; 217: 584-89. <http://dx.doi.org/10.1002/jcp.21544>
- [3] Fangusaro J. Pediatric high grade glioma: a review and update on tumor clinical characteristics and biology. *Front Oncol* 2: 105.
- [4] Jones DT, Gronych J, Lichter P, Witt O, Pfister SM. MAPK pathway activation in pilocytic astrocytoma. *Cell Mol Life Sci* 2012; 69: 1799-11. <http://dx.doi.org/10.1007/s00018-011-0898-9>
- [5] Paugh BS, Qu C, Jones C, Liu Z, Adamowicz-Brice M, Zhang J, *et al.* Integrated molecular genetic profiling of pediatric high-grade gliomas reveals key differences with the adult disease. *J Clin Oncol* 2010; 28: 3061-8. <http://dx.doi.org/10.1200/JCO.2009.26.7252>
- [6] Puget S, Philippe C, Bax DA, Job B, Varlet P, Junier MP, *et al.* Mesenchymal transition and PDGFRA amplification/mutation are key distinct oncogenic events in pediatric diffuse intrinsic pontine gliomas. *PLoS One* 2012; 7: e30313. <http://dx.doi.org/10.1371/journal.pone.0030313>
- [7] Schneider L, Clement CA, Teilmann SC, Pazour GJ, Hoffmann EK, Satir P, *et al.* PDGFRalpha signaling is regulated through the primary cilium in fibroblasts. *Curr Biol* 2005; 15: 1861-6. <http://dx.doi.org/10.1016/j.cub.2005.09.012>
- [8] Kapeller R, Chakrabarti R, Cantley L, Fay F, Corvera S. Internalization of activated platelet-derived growth factor receptor-phosphatidylinositol-3' kinase complexes: potential interactions with the microtubule cytoskeleton. *Mol Cell Biol* 1993; 13: 6052-63.
- [9] Minami A, Mizutani K, Waseda M, Kajita M, Miyata M, Ikeda W, *et al.* Necl-5/PVR enhances PDGF-induced attraction of growing microtubules to the plasma membrane of the leading edge of moving NIH3T3 cells. *Genes Cells* 2010; 15: 1123-35. <http://dx.doi.org/10.1111/j.1365-2443.2010.01450.x>
- [10] Wu C, Ramirez A, Cui B, Ding J, Delcroix JD, Valletta JS, *et al.* A functional dynein-microtubule network is required for NGF signaling through the Rap1/MAPK pathway. *Traffic* 2007; 8: 1503-20. <http://dx.doi.org/10.1111/j.1600-0854.2007.00636.x>
- [11] Stone AA, Chambers TC. Microtubule inhibitors elicit differential effects on MAP kinase (JNK, ERK, and p38) signaling pathways in human KB-3 carcinoma cells. *Exp Cell Res* 2000; 254: 110-9. <http://dx.doi.org/10.1006/excr.1999.4731>
- [12] Wang LG, Liu XM, Kreis W, Budman DR. The effect of antimicrotubule agents on signal transduction pathways of apoptosis: a review. *Cancer Chemother Pharmacol* 1999; 44: 355-61. <http://dx.doi.org/10.1007/s002800050989>
- [13] Aneja R, Dhiman N, Idnani J, Awasthi A, Arora SK, Chandra R, *et al.* Preclinical pharmacokinetics and bioavailability of noscapiene, a tubulin-binding anticancer agent. *Cancer Chemother Pharmacol* 2007; 60: 831-9. <http://dx.doi.org/10.1007/s00280-007-0430-y>
- [14] Aneja R, Asress S, Dhiman N, Awasthi A, Rida PC, Arora SK, *et al.* Non-toxic melanoma therapy by a novel tubulin-binding agent. *Int J Cancer* 2010; 126: 256-65. <http://dx.doi.org/10.1002/ijc.24765>
- [15] Naik PK, Lopus M, Aneja R, Vangapandu SN, Joshi HC. In silico inspired design and synthesis of a novel tubulin-binding anti-cancer drug: folate conjugated noscapiene (Targetin). *J Comput Aided Mol Des* 2012; 26: 233-47. <http://dx.doi.org/10.1007/s10822-011-9508-z>
- [16] Bax DA, Little SE, Gaspar N, Perryman L, Marshall L, Viana-Pereira M, *et al.* Molecular and phenotypic characterisation of paediatric glioma cell lines as models for preclinical drug development. *PLoS One* 2009; 4: e5209. <http://dx.doi.org/10.1371/journal.pone.0005209>
- [17] Valera ET, de Freitas Cortez MA, de Paula Queiroz RG, de Oliveira FM, Brassesco MS, Jabado N, *et al.* Pediatric glioblastoma cell line shows different patterns of expression of transmembrane ABC transporters after *in vitro* exposure to vinblastine. *Childs Nerv Syst* 2009; 25: 39-45. <http://dx.doi.org/10.1007/s00381-008-0740-3>
- [18] Trent J, Meltzer P, Rosenblum M, Harsh G, Kinzler K, Mashal R, *et al.* Evidence for rearrangement, amplification, and expression of c-myc in a human glioblastoma. *Proc Natl Acad Sci USA* 1986; 83: 470-3. <http://dx.doi.org/10.1073/pnas.83.2.470>
- [19] Ajeawung NF, Maltais R, Jones C, Poirier D, Kamnasaran D. Viability screen on pediatric low grade glioma cell lines unveils a novel anti-cancer drug of the steroid biosynthesis inhibitor family. *Cancer Lett* 2013; 330: 96-105. <http://dx.doi.org/10.1016/j.canlet.2012.11.034>
- [20] Ozgen U, Savaşan S, Buck S, Ravindranath Y. Comparison of DiOC6 uptake and annexin V labeling for quantification of apoptosis in leukemia cells and non-malignant T lymphocytes from children. *Cytometry* 2000; 42: 74-8. [http://dx.doi.org/10.1002/\(SICI\)1097-0320\(20000215\)42:1<74::AID-CYTO11>3.0.CO;2-6](http://dx.doi.org/10.1002/(SICI)1097-0320(20000215)42:1<74::AID-CYTO11>3.0.CO;2-6)
- [21] Woo MM, Salamanca CM, Minor A, Auersperg N. An improved assay to quantitate the invasiveness of cells in modified Boyden chambers. *In vitro Cell Dev Biol Anim* 2007; 43: 7-9. <http://dx.doi.org/10.1007/s11626-006-9002-4>
- [22] Karna P, Sharp SM, Yates C, Prakash S, Aneja R. EM011 activates a survivin-dependent apoptotic program in human non-small cell lung cancer cells. *Mol Cancer* 2009; 8: 93. <http://dx.doi.org/10.1186/1476-4598-8-93>
- [23] Dumontet C, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. *Nat Rev Drug Discov* 2010; 9: 790-803. <http://dx.doi.org/10.1038/nrd3253>

- [24] Zhou J, Giannakakou P. Targeting microtubules for cancer chemotherapy. *Curr Med Chem Anticancer Agents* 2005; 5: 65-71.
<http://dx.doi.org/10.2174/1568011053352569>
- [25] Kaverina I, Straube A. Regulation of cell migration by dynamic microtubules. *Semin Cell Dev Biol* 2011; 22: 968-74.
<http://dx.doi.org/10.1016/j.semcdb.2011.09.017>

Received on 30-04-2013

Accepted on 11-05-2013

Published on 17-07-2013

DOI: <http://dx.doi.org/10.14205/2309-3021.2013.01.01.5>

© 2013 Ajeawung *et al.*; Licensee Pharma Professional Services.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.