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Research Article

ILK/AKT Signaling Regulates miR-21 Expression in Vestibular Schwannoma and Meningioma

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Abstract

Hypothesis: Inhibition of the ILK/AKT pathway with OSU-T315 decreases miR-21 releasing the repression of miR-21 targets in vestibular schwannoma (VS) and meningioma.

Background: MicroRNAs regulate gene expression at the post-transcriptional level by binding to the 3'-untranslated region (UTR) and 5'-UTR of their targets. MiR-21 up-regulation inhibits cell death in different cancers and it is upregulated in VS. VS are caused by mutations in the neurofibromatosis type 2 (NF2) gene, and PI3K/AKT signaling drives VS tumor growth. PTEN and BTG2, among others, are targets of miR-21 and inhibitors of PI3K/AKT signaling. Therefore, miR-21 may represent a putative target for VS therapy. OSU-T315 inactivates the PI3K/AKT pathway via inhibition of the ILK-PD2 function, which indicates that OSU-T315 decreases miR-21 expression levels.

Methods: MiR-21 and its targets expression levels were analyzed by quantitative Real Time-PCR. Fluorescence in situ hybridization combined with immunofluorescence revealed miR-21 expression in VS and meningioma tumor tissues and cell lines. ILK activation by IGF-1 induction was assessed by western blots with ILK-phospho-Thr173 antibody and activated AKT by immunocytofluorescence in cell lines with the AKT-phospho-Ser473 antibody.

Results: High miR-21 levels were found in VS and meningioma cells compared to Schwann primary cells, as well as, in VS and meningioma tumor tissues. AKT inhibition with OSU-T315 decreased miR-21 levels, while increasing BTG2, PTEN, TIMP1 and PDCD4, as well as the ATG5 autophagy marker. MiR-21 inhibition with miRCURY LNA miR-21 inhibitor confirmed the repression release of the miR-21's targets expression, which results were similar to AKT inhibition. Together these data show that activated AKT up regulates miR-21 levels, repressing in this way AKT inhibitors in vestibular schwannoma and meningioma.

Conclusion: OSU-T315 decreases significantly miR-21 levels, allowing the over-expression of PTEN, BTG2, TIMP1 and PDCD4. Since OSU-T315 inactivates AKT and induces cell death by dysregulated autophagy (showing ATG5 up regulation) in VS and meningioma, these studies show that AKT regulates, in part, miR-21; and indicates that miR-21 supports tumor growth in vestibular schwannoma and meningioma by repressing AKT activation inhibitors, such as BTG2 and PTEN.

Keywords: Vestibular schwannoma; miR-21; Meningiomas

Introduction

Vestibular schwannomas (VS) are benign tumors that arise from Schwann cells of the 8th (vestibulocochlear) nerve and appear sporadically when unilateral or appear bilaterally when associated with neurofibromatosis type 2 (NF2). Patients with NF2 have germline mutations in the tumor suppressor NF2 gene, which encodes the protein merlin. VS grow slowly and progressively, causing hearing loss, tinnitus, and when large enough, brainstem compression.

Today, the therapeutic management of VS consists of microsurgery and radiotherapy [1].

However, these treatment options risk meningitis, cerebrospinal leaks, failure to control tumor growth and secondary malignancy, among others [2]. Currently, no chemotherapeutic agents to treat VS are approved by the FDA due to the lack of understanding of the molecular mechanisms that regulate VS growth.

Progress have been made in deciphering the interplay of merlin's lack of function in deregulating signaling pathways involved in cell survival and cell proliferation [1,3,4], which are well known to be active in tumor growth and cancer. Merlin suppresses the AKT signaling by forming a complex with PI 3-Kinase Enhancer-Long isoform (PIKE-L), which inhibits phosphatidylinositol 3-kinase (PI3K) activation [5,6]. Therefore, in the absence of merlin's function PI3K is activated, and active PI3K converts phosphatidylinositol

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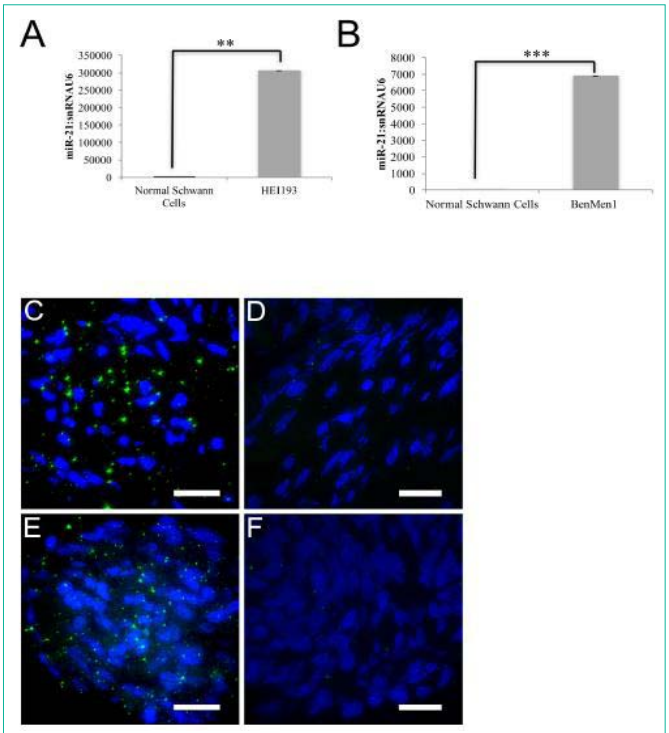

Figure 1: Vestibular schwannoma and meningioma express high levels of miR-21. A. QRT-PCR show that HEI193 and B. BenMen1 cells express significant high miR-21 expression levels compared to normal Schwann cells. C. MicroRNA fluorescence *in situ* hybridization in human FFPE vestibular schwannoma tissue section shows high expression levels of miR-21 (green). D. Vestibular schwannoma section reacted with scramble control probe and ant-digoxigenin-POD antibody. E. Human meningioma tissue section shows high miR-21 levels (green). F. Meningioma tissue section reacted with scramble control probe and secondary antibody show no staining, as expected. Expression levels of miR-21 in qRT-PCR data were normalized to snRNA-U6. Digital images obtained with DeltaVision Deconvolution microscope at 60X magnification. Scale bars represent 20 μm. Error bars represent SEM. ** p< 0.01, *** p< 0.001.

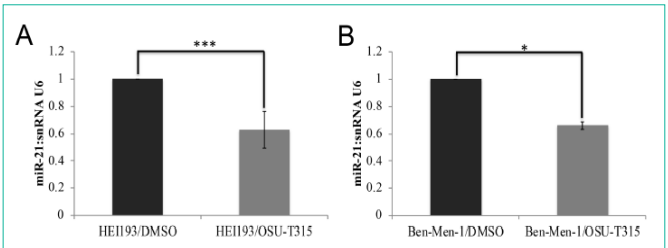

Figure 2: Inhibition of the AKT signaling with OSU-T315 decreases miR-21 expression in HEI193 and BenMen1 cells. A. Expression levels of miR-21 decrease significantly in HEI193 vestibular schwannoma cells treated with 1.8 μM of OSU-T315. B. OSU-T315 decreases miR-21 expression in BenMen1 meningioma cells with IC50 1.35 μM. Expression levels of miR-21 were normalized to snRNA-U6. Error bars represent SEM. * p< 0.05, *** p< 0.001.

(4,5)P2 (PIP2) into phosphatidylinositol (3,4,5) P3 (PIP3), which recruits AKT into the inner leaflet of the plasma membrane where PDK1 and PDK2 phosphorylates AKT at threonine 308 and serine 473, respectively [7]. Phosphorylated AKT triggers signaling for cell growth, proliferation, survival and motility, which drive tumor progression. In the normal presence of merlin function, active AKT feedback phosphorylates merlin at threonine 230 and serine 315, which abolishes merlin binding to PIKE-L; this leads to merlin degradation, increases merlin binding affinity to phosphatidylinositols and blocks its pro-apoptotic activity [5,8]. Unfortunately, the precise mechanism whereby merlin suppresses tumorigenesis remains elusive. However, a recent study reports high expression levels of micro-RNA 21 (miR-21) correlated with decreased Phosphatase and Tensin Homolog (PTEN), and hyperactivation of AKT in vestibular schwannomas [9]. These findings may open an avenue to understand further how absence of merlin function deregulates the AKT signaling in vestibular schwannoma growth.

MicroRNAs (miRNAs) are non-coding single stranded small RNAs (~ 21-23 nucleotides long) classified as tumor suppressors and oncogenes (oncomirs), which are implicated in many carcinogenic processes such as cell proliferation, cell migration, cell invasion and epithelial-mesenchymal transition (EMT), among others [10,11]. MiRNAs regulate mRNA expression by binding to the 3'UTR and 5'UTR of the target RNA, causing mRNA degradation or translational repression [12-14]. Over-expression of miR-21 in many human cancers is involved in tumor growth [12,15,16]. Studies by Cioffi, et al. have shown that blocking miR-21 expression with anti-miR-21 oligonucleotides reduces VS proliferation and induces apoptosis [9], indicating that miR-21 over-expression contributes to tumor growth and represses genes involved in cell death in vestibular schwannoma. PTEN is a tumor suppressor and a known target of miR-21 that inhibits the AKT pathway by antagonizing PI3K in reducing PIP3 to PIP2 [17]. In hepatocellular carcinoma cells, high miR-21 expression levels suppress autophagy through the AKT/PTEN pathway; and chemo sensitive studies in leukemia have shown that treatment-targeting miR-21 down regulation increases 94 the expression of autophagy related proteins such as, Beclin-1 and LC3-II [18,19]. MiR-21 interacts with a variety of genes that suppress apoptosis, sustain the proliferative signaling, participate in tumor-promoting inflammation, and activate invasion/metastasis, among other roles [20].

Recently, our group published that a small molecule inhibitor, OSU-T315, inhibits the ILK/AKT pathway by inducing autophagy in VS and meningioma cells but not by apoptosis; studies by other investigators have also shown that targeting miR-21 in leukemia cells induces autophagy [21,22]. Still, the mechanism by which miR-21 is deregulated in vestibular schwannomas is not fully understood. Here we report that miR-21 is over-expressed in VS and meningioma cell lines when compared to normal primary Schwann cells and that OSU-T315 treatment decreases the levels of miR-21 while increasing the levels of miR-21 targets downstream of AKT signaling.

These data indicate that miR-21 is, at least in part, regulated by the ILK/AKT signaling in VS and meningiomas.

Materials and Methods

Cell Culture

HEI193 and BenMen1 cell lines are human vestibular schwannoma and benign meningioma cells respectively. HEI193 cells have a mutation in the NF2 gene causing a splicing defect in the transcript but expressing moderately the active growth suppressive merlin [23]. BenMen1 cells lack one copy of chromosome 22 and the other allele has a mutation in exon 7 that causes a premature stop

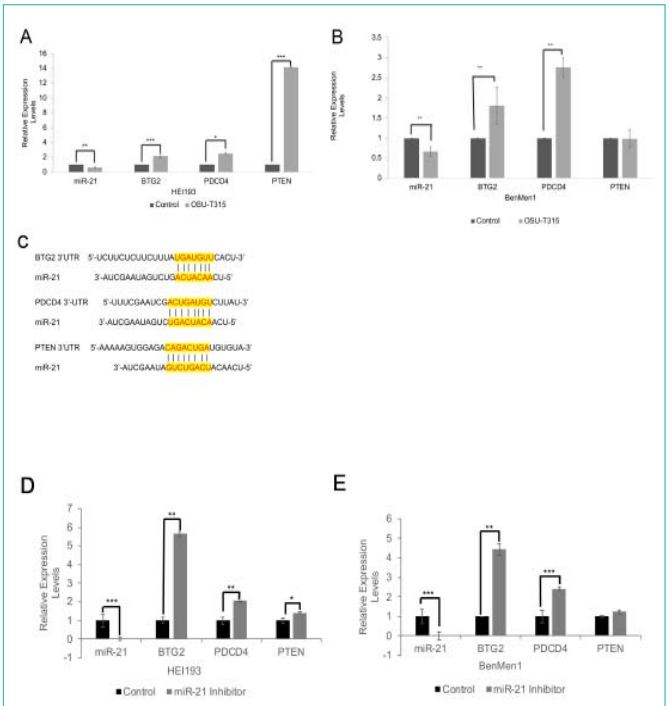

Figure 3: OSU-T315 and miR-21 inhibitors up-regulates miR-21 targets in VS and meningioma cells. A. HEI193 cells treated with OSU-T315 show decreased miR-21 expression levels, while increased expression of miR-21 targets, BTG2, PDCD4 and PTEN when compared to control basic expression levels. B. Expression levels of miR-21 decreased in BenMen1 cells treated with OSU-T315, while expression of miR-21 targets increased. C. Sequence alignment of the 3'-UTR of BTG2, PDCD4 and PTEN with the mature sequence of miR-21 show predicted binding sites of miR-21. D. QRT-PCR analysis in HEI193 and E. BenMen1 cells treated with miR-21 inhibitor show depleted miR-21 levels while increased expression of BTG2, PDCD4 and PTEN. Expression levels were normalized to GAPDH and snRNA-U6. Error bars represent SEM. * p< 0.05, *** p< 0.001.

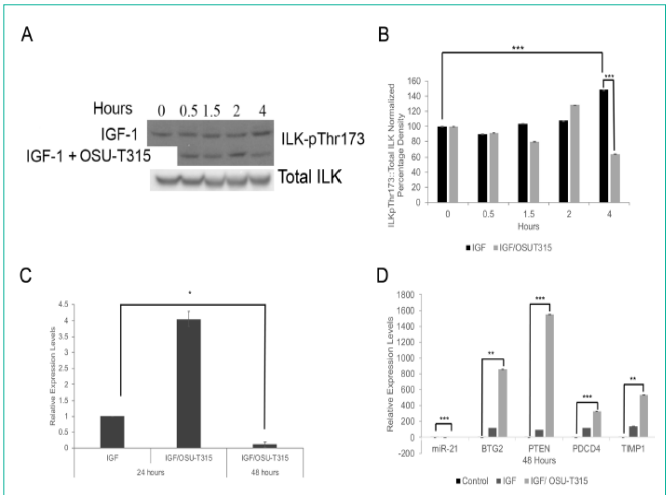

Figure 4: IGF-1 induced ILK/AKT signaling up-regulates miR-21. A. HEI193 cells show maximum ILK threonine-173 phosphorylation after 4 hours of IGF-1 induction, which decreases with OSU-T315 treatment, while total ILK is not affected. B. Densitometric measurements of western blot in A showing percentages of density signal measured. C. IGF1-induced-miR-21 expression at 24 hours is decreased significantly after 48 hours of OSUT315 treatment. D. Inhibition of miR-21 with OSU-T315 in HEI193 cells increased significantly the expression levels of BTG2, PTEN, PDCD4 and TIMP1. QRT-PCR expression levels were normalized to Beta-actin and snRNA-U6, respectively. Densitometric measurements were normalized to total ILK. Error bars represent SEM. * p< 0.05, *** p< 0.001.

codon and therefore do not express merlin isoform 1[24]. Primary Schwann cells were isolated from human femoral nerves obtained from Donor Network of Arizona (DNAZ.org).

Cell lines were plated in 100 mm plates in complete medium (DMEM high glucose supplemented with 10% FBS, 100 IU/ml penicillin-streptomycin) for the HEI193, DMEM high glucose with 10% FBS for BenMen1 (meningioma) cells, and DMEM/10% FBS, 10 ng/mL β-heregulin (R&D Systems) and 0.2 μM Forskolin (sigma) for normal primary Schwann cells. All cells were grown at 37˚ C, 5% CO2 incubation.

Treatment

OSU-T315 (Cpd22, EMD Millipore) Treatment [25]: Cells were seeded overnight at 1X10^6 cells in 100 cm plates in corresponding medium under the same conditions as described above. Next day cells were treated with IC-50 μM concentrations of OSU-T315 (HEI193: 1.8 μM and BenMen1: 1.35 μM) for 72-hours. Controls were treated with 0.01% DMSO/complete media.

IGF-1 (insulin growth factor) Induction: HEI193 cells were seeded overnight in complete medium as described above in replicas of 4. Next day after 2 hours of serum starvation (DMEM1X/2% FBS), cells were induced with 100 ng/ml of IGF-1 and treated with correspondent IC-50 of OSU-T315. Control cells were grown in complete media only. Cells were collected at different intervals of time, ½ hour, 1.5, 2, 4, 24, and 48 hours. Cell pellets were lysed for protein, mRNA, and microRNA isolation.

MicroRNA Inhibition

HEI193 and BenMen1 cells were seeded at 1X10^6 cells/plate in complete medium overnight. Next day cells were transfected separately with 50 nM of miRCURY locked nucleic acid (LNA) miRNA Inhibitors (QIAGEN, #339121 YI04100689-ADB and 339126 YI00199006-ADB), HSA-MIR-21-5p and Negative Control A. Transfection complexes were prepared with HiPerfect Transfection Reagent (Qiagen). Cells were incubated at 37° C, 5% CO_2 for 48 hours followed by isolation of microRNA and mRNA extraction.

Protein Isolation and Western blots

Cells were trypsinized and pelleted by centrifugation (1000 rpm for 5 minutes), washed with cold PBS 1X, and homogenized with RIPA buffer for 1 hour at 4˚C. Lysates were centrifuged at 4˚ C, 16,000 g for 20 min. Supernatants were isolated from pellets and 50 μg of protein were electrophoresed in 10% Polyacrylamide gels. Proteins were transferred into PVDF membranes and probed with ILK-pThr173 antibody (ThermoScientific), total ILK (ThermoScientific) and IgG-HRP (Cell Signaling).

RNA Isolation and Quantitative Real Time-PCR

RNA and miRNA isolation, cDNA synthesis and quantitative real-time polymerase chain reaction (qRT-PCR) was performed as described in Mercado-Pimentel et al., 2015 [26]. Briefly, RNA and microRNA fractions were isolated from treated and control samples with MirVana miRNA Isolation Kit (Life Technologies). cDNA syntheses of miR-21 and small nuclear RNAU6 (snRNA-U6) were

performed with specific primers [26]. QRT-PCR was performed with cDNA representing 100 ng of RNA and miR-21 expression levels were normalized to snRNA-U6 levels. Expression levels of miR-21 targets were normalized to Beta-actin or GAPDH.

Tissue Acquisition: Two cases of each, vestibular schwannoma and meningioma were acquired from the biospecimen banking and storage Arizona Cancer Center shared resource, Tissue Acquisition and Cellular/Molecular Analysis Shared Resource, supported by the National Cancer Institute Cancer Center Support Grant P30 CA023074.

Fluorescence *in situ* Hybridization and immunofluorescence

Micro-RNA in situ hybridization combined with immunofluorescence was performed according to our previous published work [27]. Briefly, digoxigenin-5'- and 3'-labeled LNA DNA probes (Exiqon) for miR-21 and scramble control were hybridized to Formalin Fixed Paraffin Embedded (FFPE) human vestibular schwannoma tissue sections of 4 µm thickness. Tissue sections were reacted with anti-digoxigenin-POD antibody (Sigma) to visualize miR-21 expression. Tyramide Signal Amplification (TSA) reaction with tyramine-conjugated fluorescein (NHS-Fluorescein) was used to visualize miR-21. Digital images were obtained with the DeltaVision Deconvolution microscope.

Immunocytofluorescence

Cells were seeded in PDLL treated coverslips at $3X10^4$ cells/well in 48-well plates overnight under cell culture conditions (see above). After 24 hours, cells were processed for immunofluorescence. Briefly, cells were fixed for 30 min with 2% PFA, permeabilized in ice for 30 min with PBDT (0.3% deoxycholate acid, 0.3% Triton X (TX)100/PBS 1X), blocked with 1%BSA/0.1% TX100/PBS1X for 1hour, incubated overnight at 4º C in primary antibody (ATG5, AKTpSer473 (Cell Signaling)), incubated at room temperature for 2 hours in secondary antibodies (Alexa Fluor 488, Alexa Fluor 568, and Alexa Fluor 647 (ThermoScientific)). Digital images were obtained using the DeltaVision deconvolution microscope.

Results

Vestibular Schwannoma and Meningioma express high miR-21 expression levels

To first investigate the relationship between miR-21 and merlin, we assessed miR-21 expression levels in cell lines with NF2 mutations having functional (HEI193) and non-functional (BenMen1) merlin versus normal primary Schwann cells. Interestingly, miR-21 levels were significantly high in both cell lines compared to normal primary Schwann cells (Figure 1A and 1B). Also, HEI193 cells had significantly higher miR-21 expression levels than BenMen1 cells. These results demonstrate that VS and meningioma cell lines with NF2 mutations express high levels of miR-21. To confirm if vestibular schwannoma and meningioma tumors express miR-21, fluorescence in situ hybridization of miR-21 was performed in 4 µm FFPE tissue sections. Both types of tumor tissue show expression of miR-21 (Figure 1C, 1D, 1E and 1F). Together, these data confirmed that vestibular schwannomas and meningiomas express significant high levels of miR-21 expression.

ILK Inhibitor, OSUT315, decreases miR-21 expression levels in vestibular schwannoma and meningioma

We have recently shown that OSU-T315 inhibits the ILK/AKT signaling pathway in VS and meningioma growth [21]; and studies by Okada have shown that active AKT inhibits merlin tumor suppressor and pro-apoptotic activities [5]. We sought to determine whether the ILK/AKT signaling pathway affected miR-21 expression levels in vestibular schwannoma and meningioma. Our published studies show that OSU-T315 inhibitor decreases cell viability after 72-hours of treatment in HEI193 and BenMen1 cells at IC50 of 1.8 µM and 1.35 µM, respectively [21]. Isolated microRNA fractions from HEI193 and BenMen1 cells treated for 72-hours with cell specific IC50 µM concentrations of OSU-T315 were analyzed by qRT-PCR for miR-21 expression levels against controls. We found that OSU-T315 decreased significantly miR-21 expression in treated cells when compared to control non-treated HEI193 and BenMen1cells (Figure 2A and 2B), indicating that miR-21 expression is regulated by the ILK/AKT signaling in vestibular schwannoma and meningioma.

Repression of mir-21 by OSU-T315 and miR-21 specific inhibitor increased miR-21 targets' expression in vestibular schwannoma and meningioma

MicroRNAs repress gene expression by binding to the 3'-UTR of their specific targets. Since vestibular schwannoma and meningioma express high miR-21 levels, repression of miR-21would increase the levels of miR-21 targets. Therefore, we hypothesized that OSU-T315 treatment would counteract the repression of miR-21 targets, B-cell Translocation Gene 2 (BTG2), programmed cell death 4 (PDCD4) and Phosphatase and Tensin Homolog (PTEN), as the miR-21 inhibitor in vestibular schwannoma and meningioma cells. BTG2 is a pan-cell cycle regulator and a tumor suppressor, which mediates the crosstalk between PIK3-AKT and NF-kB pathways [28,29]; and PDCD4 and PTEN are well known to be tumor suppressors and inhibitors of the AKT signaling activation [30,31]. Expression levels of miR-21 targets, BTG2, PDCD4 and PTEN were evaluated by qRT-PCR in OSU-T315, and separately, in miR-21 inhibitor treated cells. These data show that the miR-21 targets, BTG2 and PDCD4 increased significantly in both vestibular schwannoma and meningioma cells, while PTEN increased significantly only in HEI193 cells but not in BenMen1 cells as result of AKT inhibition (Figure 3A and 3B). Sequence alignment of the 3'-UTR sequences of BTG2, PDCD4 and PTEN show the predicted duplex formation with the mature miR-21 sequence (Figure 3C). To determine if inhibition of miR-21 with a specific LNA inhibitor affects positively the expression of these miR-21 targets in vestibular schwannoma and meningioma cells, we measured their expression levels by qRT-PCR. These data show that BTG2, PDCD4 and PTEN expression levels increased after 48 hours of inhibition compared to the controls (Figure 3D and 3E). Together, both AKT and miR-21 inhibition increased the expression levels of the miR-21 targets.

Inhibiting the IGF-induced AKT activation in vestibular schwannoma increases miR-21 target expression levels

ILK modulates the signaling of growth factors, including IGF-1, which stimulates the PI3K/AKT signaling pathway; and our published work shows that OSU-T315 inactivates AKT by inhibiting the PDK2 function of ILK in vestibular schwannoma and meningioma [32]. Therefore, to confirm if activated AKT regulates miR-21 expression, we induced ILK/AKT activation with IGF-1 in VS

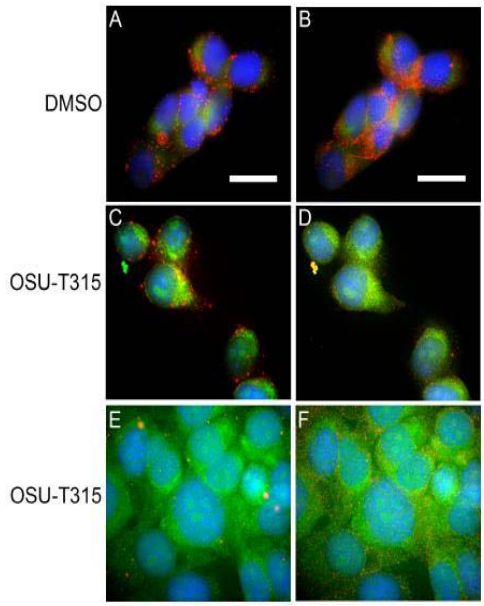

Figure 5: ATG5 levels increase in OSU-T315 treated HEI193 cells. A.DMSO control HEI193 cells express low ATG5 expression levels (green), while increased levels of miR-21 (red). B. DMSO control cells express high levels of active AKT (red: phospho-ATK-Ser473) and low levels of ATG5 (green). C. OSU-T315 treated HEI193 cells with low miR-21 expression levels (red) and increased ATG5 levels (green). D. OSU-T315 treated HEI193 cells showing very low AKT phosphorylation (red). E. OSU-T315 treated HEI193 cells reacted with scramble control probe, showing increased ATG5 levels (green). F. OSU-T315 treated HEI193 cells showing very low phosphorylated AKT levels (red) and high ATG5 expression (green). Microscope magnification is 60X, and scale bars represent 20 µm.

cells, HEI193, and then inhibited the activation with OSU-T315. Cells were collected at different time intervals to track maximum induction of ILK at threonine 173 (ILK-Thr173). Significant high ILK-Thr173 phosphorylation levels were detected at 4 hours of IGF-1 induction, which were significantly decreased with OSU-T315 (Figure 4A and 4B). However, miR-21 levels were not affected at 4 hours (data not shown) after OSUT315 treatment but rather, after 24 hours of IGF-1 induction. High IGF-1/miR-21 induced expression levels were depleted after 48 hours of OSU-T315 treatment, and expression levels of miR-21 targets increased significantly with OSU-T315 treatment, as expected (Figure 4C and 4D). These data confirm that the ILK/AKT signaling pathway regulates in part the expression of miR-21 and its targets in vestibular schwannoma; and that OSU-T315 decreases miR-21 expression, consequently inhibiting the repression of miR-21 targets by allowing their mRNA expression levels to increase.

MiR-21 expression levels decrease while ATG-5 levels increase in OSU-T315 treated VS cells

In our previous work, we demonstrated that OSU-T315 increases the levels of LC3 B in HEI193 cells [25]. LC3 lipidation is required in the process of autophagosome formation, and ATG5 conjugated to ATG12 is essential for LC3 lipidation [33-35]. To determine if ATG5 and miR-21 expression are affected in OSU-T315 treated HEI193 cells, fluorescence *in situ* hybridization combined with immuno-fluorescence and western blots were performed. These data show that OSU-T315 treatment in VS cells increases ATG5 expression levels, while decreases miR-21 and AKT phosphorylation levels in treated

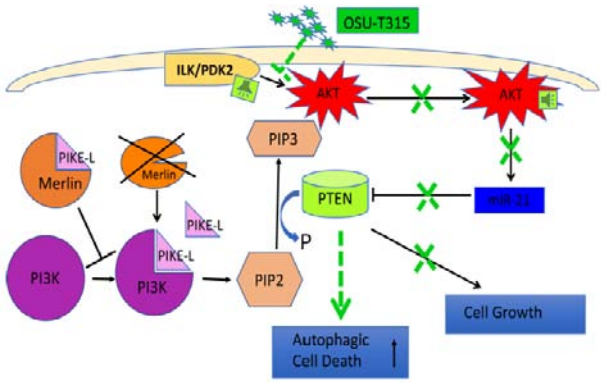

Figure 6: Suggested OSU-T315 mechanism to induce autophagic cell death in vestibular schwannoma via miR-21. OSU-T315 induces autophagic cell death in HEI193 by repressing miR-21 and allowing PTEN over expression to induce dysregulated autophagy resulting in cell death.

HEI193 versus controls (Figure 5A–5F). These data indicate that some level of autophagy takes place in HEI193, which intensifies with inhibition of the AKT pathway and consequently down-regulates miR-21. Additionally, these data confirm our previous published work that OSU-T315 induces cell death by dysregulating autophagy, and indicate that miR-21 plays a role in the process of autophagy in vestibular schwannoma.

Discussion

Our data demonstrate that compared with primary human Schwann cells, miR-21 expression is highly elevated in VS and meningioma cell lines that are known to harbor mutations in the NF2 gene. We also found that inhibition of the ILK/AKT pathway with OSU-T315 inhibitor decreases the levels of miR-21 and increase the expression of miR-21 targets, which play a role suppressing the AKT signaling pathway. MiR-21 specific inhibition resulted in similar inhibitory repression of miR-21 targets, BTG2, PTEN, and PDCD4, as in cells where AKT activation was inhibited with OSU-T315. Together, these data indicate that the ILK/AKT pathway regulates miR-21 expression in growing vestibular schwannoma and meningioma.

Our previous published work demonstrated that the ILK/AKT inhibitor, OSU-T315, induces cell death by deregulating autophagy and not by apoptosis [25]. Many recent studies have shown that the process of autophagy is controlled at different stages by specific microRNAs, including miR-21 [36]. In leukemia anti-miR-21 increased autophagy related proteins, such as Beclin-1 (ATG6), which is known to associate with activated PI3K class III in autophagosome formation [18,36]; and our data suggest that miR-21 regulates ATG5, another important player in autophagy. Additionally, studies in colon cancer have shown that increased levels of PTEN positively regulate macro-autophagy via the inhibition of the PI3K/AKT pathway; and in liver studies, PTEN expression up-regulates several ATG genes, which increases autophagy [37,38].

Taken together, our data indicate that high miR-21 expression levels deplete PTEN levels. Consequently, accumulation of PIP3 would allow the activation of the AKT signaling to support tumor growth. However, treatment of vestibular schwannomas and meningiomas

cells with OSU-T315 induces autophagy cell death by decreasing miR-21 levels. This effect halts PTEN repression causing that high PTEN levels trigger the cascade of autophagosome formation, inducing cell death by dysregulated autophagy in vestibular schwannoma and meningioma (Figure 6). Our data adds new understanding to both, molecular mechanism of miR-21 regulation, and molecular mechanism of action of OSU-T315 in vestibular schwannoma growth and cell death, respectively.

Though the study field of these benign tumors, vestibular schwannoma and meningiomas, have limitations concerning the availability of cell lines and tumor tissues due to the low incidence of these tumors, our study confirms miR-21 expression in tumor tissues. These data show a strong evidence of the role of miR-21 in VS and meningiomas. Furthermore, our data deciphers a substantiated suggested mechanism of miR-21 regulation, and a door to consider miR-21 as a putative biomarker for drug development to treat afflicted patients with these tumors.

Funding

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