Video Article

Induction of Mesenchymal-Epithelial Transitions in Sarcoma Cells

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Abstract

Phenotypic plasticity refers to a phenomenon in which cells transiently gain traits of another lineage. During carcinoma progression, phenotypic plasticity drives invasion, dissemination and metastasis. Indeed, while most of the studies of phenotypic plasticity have been in the context of epithelial-derived carcinomas, it turns out sarcomas, which are mesenchymal in origin, also exhibit phenotypic plasticity, with a subset of sarcomas undergoing a phenomenon that resembles a mesenchymal-epithelial transition (MET). Here, we developed a method comprising the miR-200 family and grainyhead-like 2 (GRHL2) to mimic this MET-like phenomenon observed in sarcoma patient samples.We sequentially express GRHL2 and the miR-200 family using cell transduction and transfection, respectively, to better understand the molecular underpinnings of these phenotypic transitions in sarcoma cells. Sarcoma cells expressing miR-200s and GRHL2 demonstrated enhanced epithelial characteristics in cell morphology and alteration of epithelial and mesenchymal biomarkers. Future studies using these methods can be used to better understand the phenotypic consequences of MET-like processes on sarcoma cells, such as migration, invasion, metastatic propensity, and therapy resistance.

Video Link

The video component of this article can be found at https://www.jove.com/video/55520/

Introduction

Phenotypic plasticity refers to a reversible transition between cellular phenotypes, and is commonly divided into two types, epithelial-to-mesenchymal (EMT) transitions and mesenchymal-to-epithelial transitions (MET). This phenotypic plasticity plays an important role in normal processes of multicellular organisms, such as development and wound healing[1]; however, these same pathways and gene expression programs can also lead to disease, such as fibrosis (reviewed in[2,3,4]) and carcinoma metastasis (reviewed in references[5,6,7,8]). During metastasis, for example, EMT disrupts cell polarity, cell-cell interactions, and promotes invasion[9,10]. Together, EMT contributes to a phenotypic state that facilitates cancer cell dissemination. In addition, EMT also leads to a host of other phenotypic alterations that drive an aggressive phenotype, including deregulation of cancer cell metabolism[6], development of drug resistance[11,12], increased tumor-initiation ability[13,14] and host immune evasion[15].

Phenotypic plasticity has been well studied in carcinoma progression; however, sarcomas also exhibit phenotypic plasticity. Interestingly, it appears as if some of the same drivers of phenotypic plasticity in carcinomas also contribute to sarcoma plasticity and aggressiveness. For instance, circulating tumor cells (CTCs) from sarcoma patients have been shown to express EpCAM, a cell surface protein that is typically found on epithelial cells[16]. Additionally, 250 soft tissue sarcoma samples were categorized as epithelial-like or mesenchymal-like based on gene expression. Patients in the epithelial-like biomarker signature had a better prognosis than patients with the mesenchymal-like biomarker signature[17]. This is consistent with many carcinomas, in which patients with more epithelial-like carcinomas have better outcomes compared with patients with more mesenchymal-like tumors[18].

While some sarcomas display biomarkers and gene expression pathways consistent with MET, the molecular underpinnings of this phenotypic plasticity remain poorly understood. To study the mechanisms and drivers of MET in sarcoma we developed a model of MET induction using two epithelial-specific factors, the microRNA (miR)-200 family and grainyhead-like 2 (GRHL2). The miR-200s are a family of small non-coding RNAs that regulate gene expression by binding to the 3' UTRs of messenger RNA and preventing translation into protein. The miR-200 family consists of two subgroups - one containing miR-141 and miR-200a, and the other including miR-200b, miR-200c, and miR-429. Members of the miR-200 family are enriched in epithelial tissues, and the loss of miR-200s is associated with metastasis in carcinomas[19]. The miR-200 family

is also downregulated in soft tissue sarcomas compared to normal tissue[20]. Similar to the miR-200s, GRHL2 is a key regulator that is important for epithelial development[21]. The GRHL2 transcription factor acts in two ways to upregulate epithelial genes, such as E-cadherin: 1) In epithelial cells, GRHL2 directly represses the EMT master regulator, ZEB1[22]; and 2) GRHL2 directly activates transcription of epithelial genes[23]. Our previous investigations have shown that combined expression of miR-200s and GRHL2 in sarcoma cells induces an MET-like phenotype[24]. Here, we present a detailed protocol to create an *in vitro* model of MET induction in sarcoma cells using ectopic expression of miR-200s and GRHL2.

Protocol

1. Preparation of Reagents

1. Prepare DMEM for cell culture by adding 50 mL of fetal bovine serum (FBS) and 5 mL of penicillin-streptomycin (5,000 U/mL) to 500 mL of DMEM. This medium can be stored at 4 °C for up to six months.
2. Resuspend lyophilized primers in nuclease-free water to a final concentration of 10 µM. Store re-suspended primers at -20 °C.
3. Prepare radioimmunoprecipitation assay (RIPA) buffer (150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0). Supplement with protease inhibitor cocktail before use and keep buffer on ice when in use. Store at 4 °C.
4. Prepare 10 mg/mL polybrene (hexadimethrine bromide) in water and syringe filter to sterilize using a 0.45 µm polyethersulfone filter. Solution can be stored at 4 °C for up to six months.
5. Prepare a 1 mg/mL working solution of nitro blue tetrazolium chloride by dissolving 10 mg in 10 mL of PBS. Store at 4 °C.

2. Lentiviral Transduction of GRHL2

Day 1

1. Plate 3 x 10^5 HEK293T cells per well of a 6-well plate in 2 mL of supplemented DMEM. Quantify cells using an automated cell counter. Cells should be 40 - 60% confluent after overnight culture at 37 °C with 5% CO_2.

Day 2

2. Dilute 2 µg of empty vector (pCMV-UBC-EGFP) or 2 µg of pCMV-GRHL2-UBC-EGFP[24,25] in 200 µL of serum-free media along with 1.8 µg of pΔ8.9 and 0.2 µg of pCMV-VSV-G helper plasmids. In a separate tube, dilute 4 µL of a lipid-based transfection reagent in 200 µL of serum-free media per transfection (8 µL in 400 µL for two samples). Add 200 µL of transfection reagent mixture to each plasmid solution and incubate for 20 min at room temperature.
3. During the incubation, wash HEK293T cells by removing the medium by vacuum aspiration and rinsing cells with 1 mL of PBS. Replace the PBS with 800 µL of serum-free media. After 20 min, add 200 µL of transfection reagent:plasmid mixture from step 2.2 dropwise to each well and incubate cells for 2 h at 37 °C in 5% CO_2.
4. Carefully remove transfection medium by vacuum aspiration and replace with 2 mL of supplemented DMEM. Return the cells to the incubator for overnight culture.
   NOTE: HEK293T cells are loosely adherent. Media replacement must be performed with caution to limit removal of cells from the bottom of the dish or flask.

Day 3

5. Refresh media on transfected HEK293T cells and plate 3 x 10^5 RD cells per well of a 6-well plate in 2 mL of supplemented DMEM. Culture cells overnight. RD cells are a commercially-available human rhabdomyosarcoma cell line.

Day 4

6. Collect viral media from HEK293T cells. Pipette the DMEM media from the HEK293T cells and place into a new 15 mL conical. Carefully replace with new DMEM media and place HEK293T cells back in the incubator for the next day.
7. Add 2 µL of 10 mg/mL polybrene per mL of viral media into two new 50 mL conical tubes (one for the empty vector transfection and another for the GRHL2 transfection). Syringe filter viral media by removing the plunger from a 3 mL syringe and attach a 0.45 µm polyethersulfone filter to the tip.
   1. Add the viral media collected in step 2.6 into the barrel of the syringe, and plunge media into the new 50 mL conical tubes containing polybrene. Remove media from RD cells by vacuum aspiration and add filtered viral media to RD cells.

Day 5

8. Repeat Day 4. HEK293T cells can be discarded after the viral media has been collected.

Day 7

9. Wash RD cells with 1 mL of PBS and add 200 µL of 0.05% trypsin per well. Incubate at 37 °C for 5 min, add 2 mL of supplemented media, and move cell suspension to a new 15 mL conical. Centrifuge cells at 250 x g for 5 min at room temperature and aspirate media. Resuspend in 1 mL of DMEM (supplemented with 5% FBS and 1% penicillin-streptomycin).
   NOTE: Using a higher percentage of FBS may cause clogging during flow cytometry
   1. Filter cells for flow cytometry. Use a pipette to apply the 1 mL RD cell suspension through a 30 µm filter into flow cytometry tubes and place tubes on ice.

2. Sort EGFP+ cells[26] into 1.5 mL microcentrifuge tubes containing 0.5 mL of DMEM supplemented with 10% FBS and 1% penicillin-streptomycin and place on ice. Plate EGFP+ sorted cells in total of 1 mL of supplemented DMEM into a single well of a 12-well plate and place in the incubator for culture.
   NOTE: The yield of EGFP+ cells from flow cytometry after this transduction is usually low (50,000-100,000 cells). Cells may need to be cultured for 10 - 14 days before proceeding to step 3.

3. Reverse Transfection of miR-200s

1. Prepare 50 µM stocks of miR-200 mimics using nuclease-free H_2O. Aliquot stocks and store at -20 °C to avoid repeated freeze/thaw cycles.
2. Add 3 µL of each 50 µM miR-200 mimic (miR-200a, miR-200b, and miR-200c) to 300 µL of serum-free media or 9 µL of 50 µM negative control miRNA to 300 µL serum-free media.
3. Add 6 µL of siRNA-specific transfection reagent to 600 µL of serum-free media and divide 300 µL of this mixture into two tubes, one for each of the two miR mixtures from step 3.2. Combine the 300 µL of each miR mixture from step 3.2 with a mix of 300 µL of transfection reagent. Incubate for 20 min at room temperature.
4. While incubating, prepare a cell suspension of 600,000 EGFP+ RD cells expressing EV or GRHL2 created in Section 2 in 2.4 mL (250 cells/µL) of serum-free media per treatment.
5. In a 24-well plate, add 100 µL per well of miR-200 mix or negative control mix from step 3.3 into six wells, and then add 400 µL of each cell suspension into three wells of each miR mix.
6. Incubate cells at 37 °C in 5% CO_2 overnight and change media to fully supplemented DMEM the following day. Collect cells 2 days later for analysis using the appropriate buffer (see below). Time-lapse imaging of RD sarcoma cells undergoing MET is available as supplemental material. Image each well every 2 h with a 10X objective using an automated live-cell imager[27].

4. RNA extraction, Reverse transcription, and qPCR

1. Extract RNA according to manufacturer's instructions using a standard RNA extraction kit[24]. Extracted RNA can be stored at -80 °C.
2. Quantify RNA concentration. Thaw and work with RNA on ice. To quantify RNA concentrations, measure UV absorbance at 260 nm with a plate reader spectrophotometer according to the manufacturer's protocol. Dilute all samples to the lowest concentration with nuclease-free water.
3. Perform reverse transcription of total RNA into complementary DNA (cDNA). Combine no less than 100 ng of total RNA with PCR buffer, dNTP mix (100 mM), random hexamer primers, reverse transcriptase and nuclease-free water in a 20 µL reaction volume[24]. Run RT cycles according to the manufacture's protocol. cDNA can be stored long-term at -20 °C.
4. Dilute 20 µL reactions to 100 µL with 80 µL of nuclease-free H_2O.
5. Mix 5 µL of a fluorescence-based intercalating dye 2x qPCR master mix, 0.06 µL of each 10 µM primer, and 2 µL of diluted RT reaction per sample.
6. Run qPCR according to manufacturer's protocol for the fluorescence-based master mix.
7. Quantify relative amounts of mRNA by the delta CT method and normalize to GAPDH. Plot the mean mRNA expression of biological replicates ± standard deviation for each treatment group.
   NOTE: Special precautions should be taken when working with RNA to avoid contact with RNases, such as using RNase-free plastics and reagents. Non-disposable equipment should be treated before use to remove RNases.

5. Immunofluorescence Staining

1. Following step 3.6, aspirate media by vacuum aspiration from RD cells in 24-well format.
2. Fix cells by adding 500 µL of 4% paraformaldehyde (PFA) per well and incubate for 15 min at room temperature (RT). Following fixation, place cells in phosphate buffered saline (PBS) and store at 4 °C overnight if necessary.
3. Permeabilize cells by adding 500 µL of PBS+0.2% Triton-X100 and incubate 30 min at RT. By vacuum aspiration, remove permeabilization buffer and wash three times with PBS.
4. Block in 500 µL of 5% bovine serum albumin (BSA)/PBS and incubate cells for 30 min at RT. Cells can be stored at 4 °C overnight if necessary.
5. Prepare primary antibody dilution. Pipette 200 µL of 5% BSA/PBS per well into a 15 mL conical (12 wells = 2.4 mL) and add 1 µL of primary antibody per mL of 5%BSA/PBS needed. Remove blocking buffer by vacuum aspiration and dispense 200 µL of diluted primary antibody to each well.
   1. Incubate cells in 1:1,000 diluted primary antibodies in 5% BSA/PBS for 1 h at RT or overnight at 4 °C. After incubation, wash cells two times with PBS.
6. Prepare the secondary antibody dilution in the same volume of 5% BSA/PBS as above. Add the far-red dye-conjugated secondary antibody using a 1:2,000 dilution. Additionally add 1 µg/mL of Hoechst dye to the mix. Remove the PBS and add 200 µL of the mix to each well.
   1. Incubate at RT for 1 h in the dark. Wash 3 times with PBS, leave cells in PBS, and protect cells from light using foil. Cells can be stored at 4 °C if necessary.
7. Image cells at 400X total magnification on an inverted epifluorescence microscope with excitation wavelength 594 - 650 nm.

6. Western Blotting

1. Wash cells from 3.6 twice with ice-cold PBS. On ice, lyse cells with 50 µL of 1x RIPA buffer supplemented with 1x protease inhibitor cocktail.
2. Rock lysates at 4 °C for 15 min, collect lysates into 1.5 mL tubes, and centrifuge at high speed (20,000 x g) for 5 min to clarify samples. Cell lysates can be stored long-term at -80 °C if necessary.

3. Quantify total protein using a Bradford assay and aliquot an equal amount of protein into new 1.5 mL microcentrifuge tubes. Bring samples to equal volume using RIPA buffer and 3x Laemmli loading buffer supplemented with 2-mercaptoethanol.
4. Incubate samples in 1x Laemmli sample loading buffer for 5 min at 95 °C and run SDS-PAGE using a 4-12% Tris-glycine gel at 200 V for 45 min. The amount of protein loaded ranges depending on the cell line. In some cases, 50-100 µg of total protein is needed to detect E-cadherin in mesenchymal cell lines.
5. Transfer proteins onto a nitrocellulose membrane for 2 h at 50 V in 1x Tris-glycine transfer buffer.
6. Block membrane for 1 h at room temperature or overnight at 4 °C in a BSA-based blocking buffer.
7. Dilute primary antibodies at 1:1,000 concentration in 5 mL of blocking buffer, add dilution to the membrane, and incubate with gentle rocking for 1 h at room temperature or overnight at 4 °C. Wash membrane 3 times in PBS with 0.05% Tween-20.
8. Incubate membranes for 1 h at RT with infrared fluorescent-coupled secondary antibody at 1:20,000 dilution in blocking buffer as above.
9. Wash membrane two times in PBS with 0.05% Tween-20 and then one time in PBS.
10. Image membrane using standard infrared fluorescence detection.

7. Anchorage-independent Growth Assays

NOTE: For a detailed soft agar assay protocol, see [28].

1. Warm sterile 2x DMEM media to 42 °C in hot water bath. During this time, heat pre-autoclaved 1% agarose in the microwave for 3 min. Microwave for an additional 30 s at a time as needed or until completely melted. Move agarose to a 42 °C water bath.
2. Place a 50 mL conical tube in a beaker with 42 °C water in a sterile hood and mix 1% agarose and 2x DMEM media in a 1:1 ratio accounting for 1.5 mL of mixture per well in a 6-well plate. Always prepare extra DMEM/agarose to account for pipetting error and to avoid bubbles.
3. Add mixture to the sides of the well, ensuring no bubbles form and let stand for 30 min at RT.
4. While the bottom layer is solidifying, prepare each group of RD cells transfected in step 3 by aspirating media and washing once with 1 mL PBS. Aspirate PBS and add 0.2 mL of 0.05% trypsin and incubate at 37 °C for 5 min.
5. Neutralize trypsin in 0.8 mL of media and triturate cells to form a single-cell suspension. Transfer cell suspension to a 15 mL conical tube.
6. Count cells and calculate volume of cell suspension required for 10,000 cells per well.
7. Heat 0.6% agarose in the microwave for 3 min followed by 30 s at a time until completely melted. Move agarose to 42 °C water bath.
8. Place a 50 mL conical tube in a beaker with 42 °C water in a sterile hood. Mix 0.6% agarose and diluted cell suspension in a 1:1 ratio to prepare 1.5 mL of mixture per well in a 6-well plate. Always prepare extra cell suspension/agarose mix to account for pipetting error and to avoid bubbles.
   NOTE: It is important to work at 42 °C here. If the temperature is too low the mixture will solidify before plating, and temperatures above 42 °C will affect cell viability.
9. Mix well by triturating cells several times and add cell mixture to the wells, ensuring no bubbles form, and let solidify for 20 min at RT.
10. **Add 2 mL of supplemented media to each well and move plates to incubator.**
    1. Change media once a week for 3-4 weeks or until multi-cellular colonies form. Be careful to avoid touching the agar when removing media by vacuum aspiration. Alternatively, use a P1000 to remove the top layer of liquid media.
    2. Stain soft agar plates by adding 200 µL of Nitro Blue Tetrazolium chloride solution (1 mg/mL in PBS) and incubate the plate overnight at 37 °C. Replace media the next day with PBS for imaging.
    3. Image wells at 40X total magnification on an inverted microscope.
    4. Perform analysis on ImageJ for colony area and number. Plot mean colony number of biological replicates ± standard deviation.

Representative Results

Schema for MET induction in sarcoma cells

A general timeline for the induction of MET-like changes in sarcoma cells is shown in **Figure 1**. The protocol begins by transducing GRHL2 (**Figure 1A**), followed by transfection of the miR-200 family (**Figure 1B**). GRHL2 or miR-200 family members were not able to impact the appearance of RD cells when expressed alone, but ectopic expression of GRHL2 and miR-200s together results in epithelial-like morphological changes in RD cells. Cells transition from a spindle-shaped form to a more rounded appearance with increased cell-cell contact (**Figure 1C**).

Induction of MET-like changes in sarcoma cells

The morphological change in RD cells upon GRHL2 and miR-200 over-expression was accompanied by upregulation of the epithelial marker E-cadherin (**Figure 2A**). Addition of miR-200s upregulated E-cadherin alone, but combined miR-200s and GRHL2 overexpression synergistically enhanced E-cadherin expression (**Figure 2A**; not log scale). In addition, there was an increase at cell-cell junctions of epithelial adhesion molecules, EpCAM and TJP1, white arrows (also known as zona occludens 1, ZO-1) (**Figure 2B**).

MET induction decreases the colony formation ability of sarcoma cells

Upregulation of epithelial proteins was accompanied by downregulation of mesenchymal genes Zeb1 and Notch1 (**Figure 3A**), which are known targets for miR-200. Induction of MET reduced the anchorage-independent growth of RD cells as measured by colony number (**Figure 3B**). This growth inhibition was driven by miR-200s alone; as overexpression of GRHL2 led to an increase in anchorage independent growth (**Figure 3**). This is consistent with previous reports showing GRHL2 expression induces anchorage-independent growth[22].

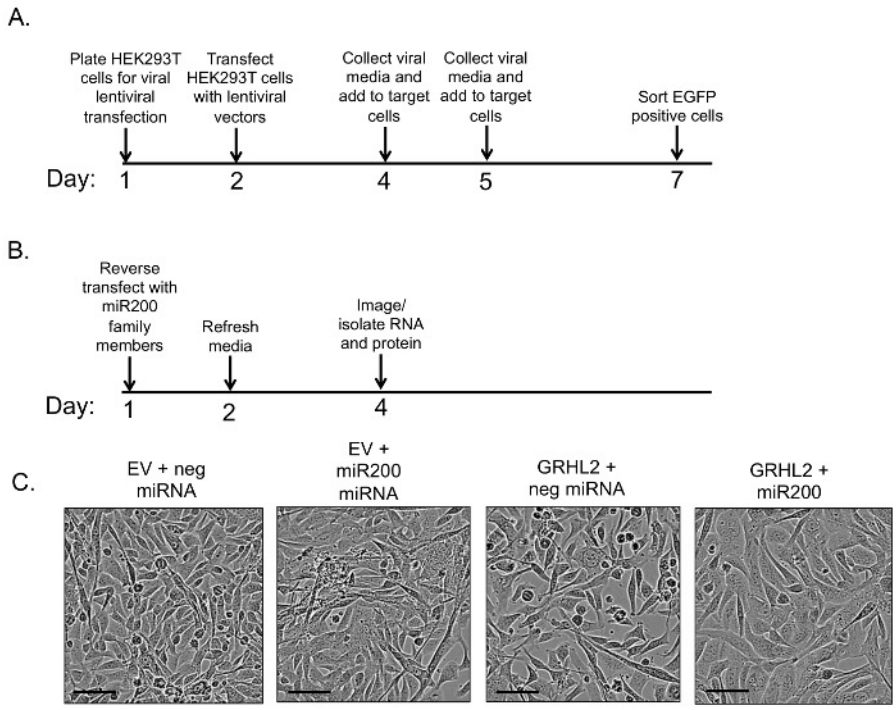


Figure 1: Timeline of MET Induction with GRHL2 Over-expression and miR-200 Transfection. (**A**) Timeline of ectopic over-expression of GRHL2 in target cells. (**B**) Timeline of MET induction *via* reverse transfection of miR-200 family members in target cells. (**C**) GRHL2 and miR-200 over-expression led to changes in morphology of target cells consistent with MET. Scale bar = 75 µm Please click here to view a larger version of this figure.

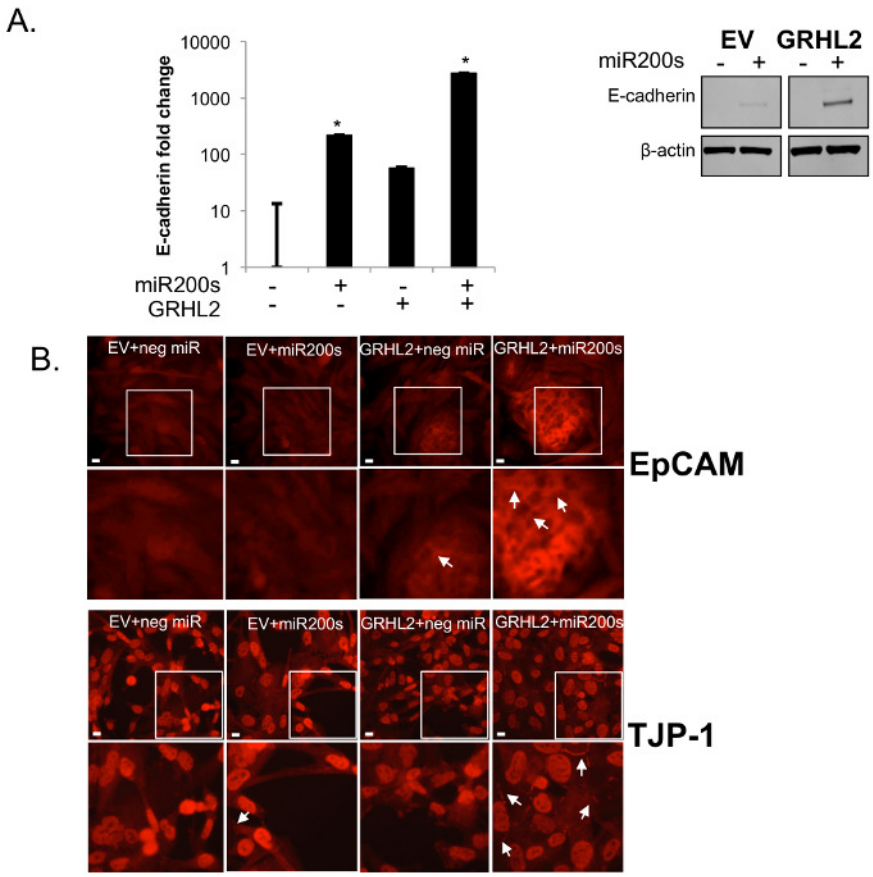


Figure 2: Concurrent Over-expression of GRHL2 and miR-200s Led to MET in Target Cells. (**A**) Expression of miR-200s led to elevated E-cadherin expression while combined expression of GRHL2 and miR-200s produced a synergistic effect on E-cadherin expression at both the mRNA (mean values ± standard deviation) and protein levels. (**B**) Combined expression of GRHL2 and miR-200s led to increased expression of EpCAM and TJP1 at cell-cell contacts (arrows). Scale bar = 20 µm. * indicates $p < 0.05$ analyzed using ANOVA with Tukey's post-hoc correction.This figure has been modified from reference[24]. Copyright © American Society for Microbiology, *Molecular Cell Biology*, volume 36, Issue 19, 2503-2513, 2016. Please click here to view a larger version of this figure.

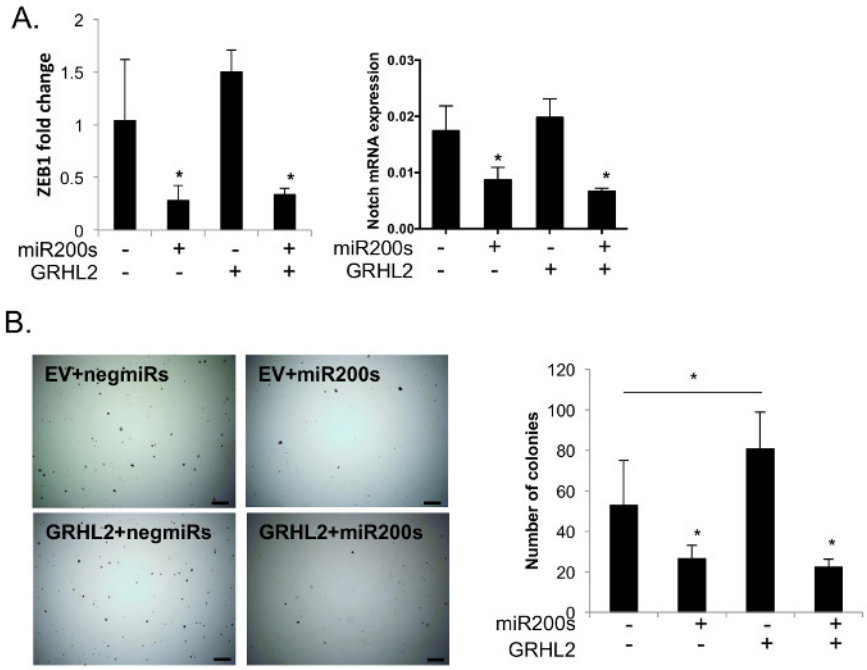
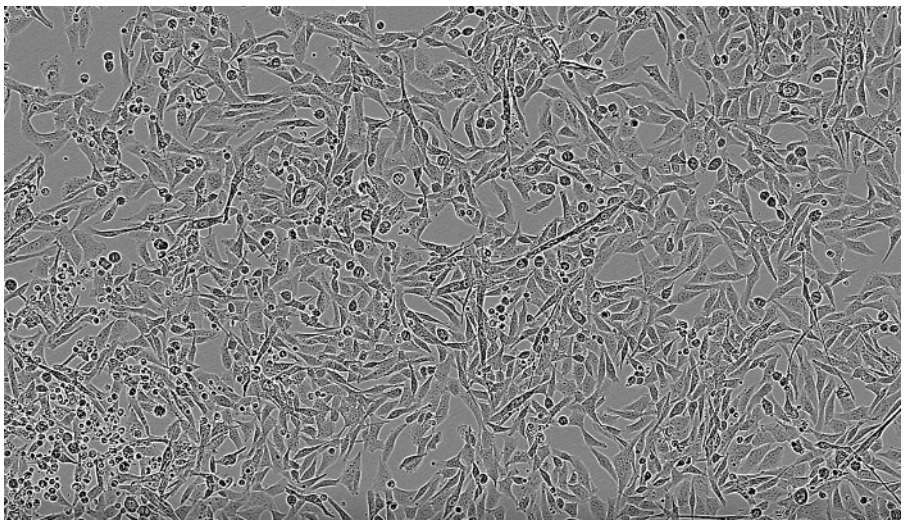
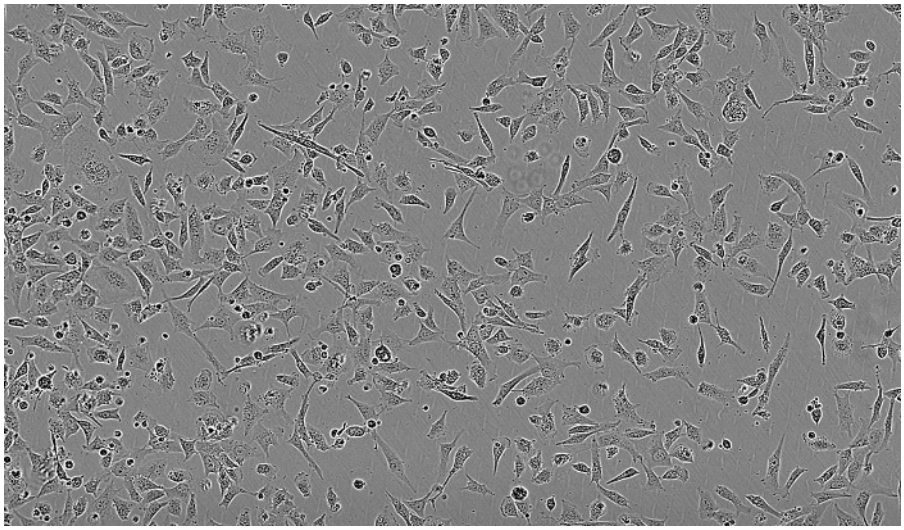


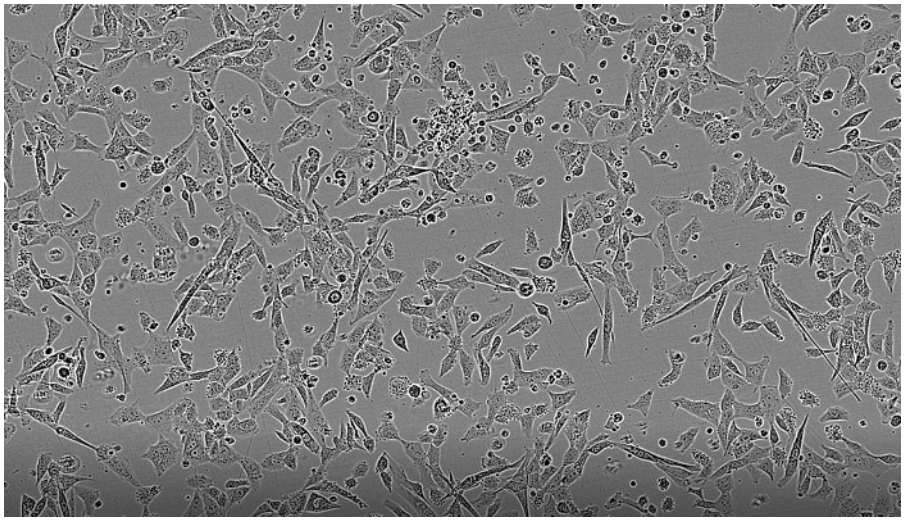
Figure 3: Expression of miR-200s Suppresses Mesenchymal Markers and Decreases Anchorage-independent Growth in Sarcoma Cells. (**A**) Over-expression of miR-200s but not GRHL2 inhibited Zeb1 and Notch1 mRNA expression (mean values ± standard deviation). (**B**) Over-expression of miR-200s but not GRHL2 inhibited anoikis resistance in sarcoma cells. Representative images of stained colonies (Scale bar = 200 µm) and quantification of colony number (mean values ± standard deviation) are shown. * indicates $p < 0.05$ analyzed using ANOVA with Tukey's post-hoc correction.This figure has been modified from reference[24]. Copyright © American Society for Microbiology, *Molecular Cell Biology*, volume 36, Issue 19, 2503-2513, 2016. Please click here to view a larger version of this figure.



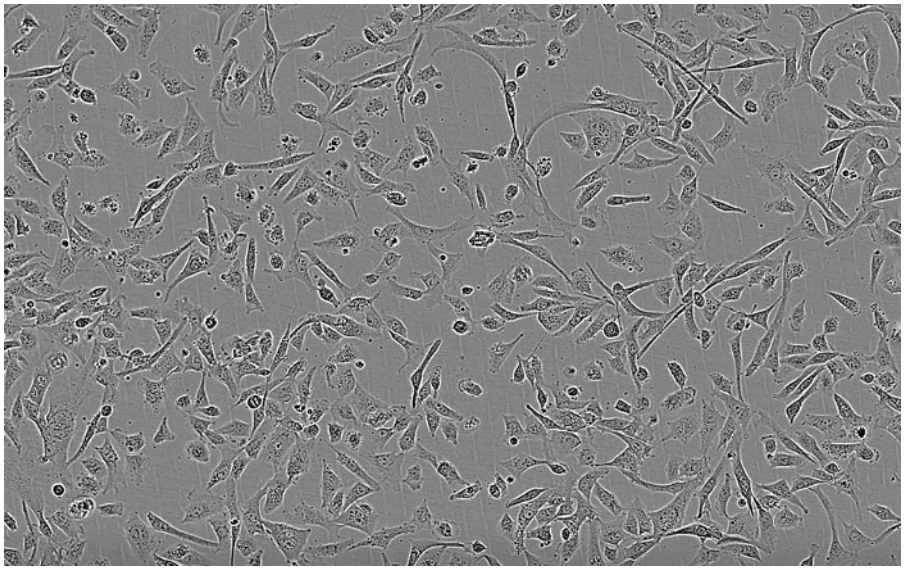
Supplemental Video : RD cells expressing empty vector and negative control miRNAs. Videos were compiled from images of RD cells transfected with empty vector and negative control miRNAs acquired every two hours using an automated live-cell imager. Please click here to view this video. (Right-click to download.)



Supplemental Video 2: RD Cells Expressing GRHL2-EGFP and Negative Control miRNAs. Videos were compiled from images of RD cells transfected with GRHL2-EGFP and negative control miRNAs acquired every two hours using an automated live-cell imager. Please click here to view this video. (Right-click to download.)



Supplemental Video 3: RD Cells Expressing Empty Vector and miR200 miRNAs. Videos were compiled from images of RD cells transfected with empty vector and miR200 miRNAs acquired every two hours using an automated live-cell imager. Please click here to view this video. (Right-click to download.)



Supplemental Video 4: RD Cells Expressing GRHL2-EGFP and miR200 miRNAs. Videos were compiled from images of RD cells transfected with GRHL2-EGFP and miR200 miRNAs acquired every two hours using an automated live-cell imager. Please click here to view this video. (Right-click to download.)

Discussion

Sarcomas are rare, but highly aggressive cancers of a mesenchymal lineage. Despite their mesenchymal lineage, a subset of sarcomas appears to undergo a phenotypic transition to a more epithelial-like state. This MET-like switch has prognostic relevance, as patients with more epithelial-like tumors are less aggressive[24]. Despite their clinical relevance, there are few studies addressing the molecular mechanisms driving these phenotypic transitions in sarcomas.

To examine MET-like transitions in sarcoma cells, we have developed an MET-induction model by combining expression of epithelial factors GRHL2 and the miR-200 family. This method rapidly induces sarcoma cells to become more epithelial-like as measured by alterations in morphology and gene expression. Using this protocol to induce MET in sarcoma cells facilitates study of the impact of these transitions on the phenotypes that drive sarcoma aggression, such as migration, invasion, proliferation and death resistance and how each of these changes in biology can affect drug resistance.

In the context of epithelial-derived carcinomas, expression of one mesenchymal factor is often sufficient to induce EMT[14]. However, in this sarcoma-derived model of MET the expression of two epithelial factors, GRHL2 and miR-200s, are required. Interestingly, the miR-200s alone had a stronger effect on most biomarkers of MET than GRHL2, while GRHL2 was able to robustly activate epithelial genes only in the presence of miR-200-based repression of epithelial gene repressors, such as ZEB1[24]. It is possible that for some mesenchymal cell types epithelial genes need to be both de-repressed (*e.g. via* miR-200s) and activated (*e.g. via* GRHL2) to drive MET.

We have experienced variation in the levels of GRHL2 expression across different cell lines. To overcome this, we used a GRHL2 expression plasmid that also expresses EGFP[25] to sort by flow cytometry EGFP positive cells prior to experiments. It is also critical to validate the functionality of miR-200s and GRHL2 in different cell types. EMT is viewed as a spectrum based on expression of epithelial and mesenchymal-associated genes, which can have context-dependent variation based on cell line, cancer type, treatment, etc. Therefore, we analyzed five genes regulated by miR-200 or GRHL2 to account for cell context-dependent changes. Another caveat of this assay is the reliance on a transient transfection of miR-200s for MET induction. Thus, long-term experiments can become costly and complicated when multiple repeat transfections become necessary. This can be overcome by using miR-200 expression plasmids.

Other studies have also reported evidence of MET in sarcomas. For instance, MET was observed in a subset of leiomyosarcomas and was associated with better survival for patients. Mechanistically, Yang et al. found that in a leiomyosarcoma cell line inhibition of Slug with siRNA was sufficient to induce MET-like changes[29]. Likewise, depletion of yet another mesenchymal factor, Snail, in mesenchymal stem cells reduced sarcoma formation in mice[30]. Thus, it is clear from the literature that there are multiple pathways to an MET-like phenotype, which may vary by cell type. While this is not the only way to induce MET, we have used our method to induce MET in two sarcoma subtypes, including rhabdomyosarcoma (RD cells) and osteosarcoma (143B cells). In the future, it would be interesting to compare these different methods in a broader range of sarcoma cells. For example, do certain sarcoma subtypes have underlying genetic or epigenetic alterations that make them more or less susceptible to MET induction?

Identifying the impact of MET on a variety of biological outputs in sarcomas cells could provide a better understanding of why patients with more epithelial-like sarcomas have an improved prognosis. In addition, understanding the impact of treatment on driving the phenotypic transitions between states would deepen our biological understanding and inform upon response to current therapies in sarcoma patients.

Disclosures

The authors have nothing to disclose.

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