TITLE: To study the role of fodrin, a non erythroid spectrin, in cell cycle and mitosis.

A thesis submitted in partial fulfilment of the requirements for the BS-MS dual degree programme in IISER Pune.

Submitted by: Anu. S. R

Reg. no. 20111034.

Biology division

Indian Institute of Science Education and Research Pune.

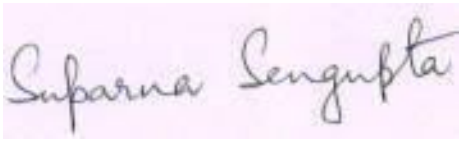
Supervisor: Dr. Suparna Sengupta (Scientist E Ⅱ, Cancer Research, Rajiv Gandhi Centre for Biotechnology)

Thesis Advisor: Dr. Mayurika Lahiri (Associate Professor, Department of Biology, IISER Pune)

Institute where the project has been done: Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

CERTIFICATE.

This is to certify that this dissertation entitled "**To study the role of fodrin, a non erythroid spectrin, in cell cycle and mitosis**" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by "**Anu. S. R** at **Rajiv Gandhi Centre for Biotechnology**" under the supervision of "**Suparna Sengupta, Scientist E Ⅱ, Cancer Research**" during the academic year **2016-2017**.



Dr. Suparna Sengupta
Scientist E Ⅱ,
Cancer Research,
Rajiv Gandhi Centre for Biotechnology
Thiruvananthapuram.

Thiruvananthapuram,
20-03-17.

DECLARATION.

I, **Anu. S. R** hereby declare that the matter embodied in the report entitled "...**To study the role of fodrin, a non erythroid spectrin, in cell cycle and mitosis.**....." are the results of the work carried out by me at the Department of **Cancer Research**, **Rajiv Gandhi Centre for Biotechnology**, under the supervision of **Dr. Suparna Sengupta** and the same has not been submitted elsewhere for any other degree.

Anu. S.R,
Reg. no: 20111034,
BS MS Student,
Indian Institute of Science Education and Research, Pune.
20-03-2017

Thiruvananthapuram,
20-03-17.

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Finally I thank my parents, brother and friends for their support and motivation.

ABSTRACT.

Fodrin or non erythroid spectrin is composed of an α (240kDa) and a β (235kDa) subunit that form a tetrameric complex. It was isolated from goat brain as a part of cytoplasmic gamma tubulin ring complex, which is the nucleating agent of microtubules in mammalian cells. Gamma tubulin is present in cytoplasm as well as centrosomes but it is from the centrosome that gamma tubulin ring complex nucleates microtubules. The nucleation ability increases manifold for mitotic cells compared to interphase cells. In order to study the role of fodrin on HEK293 cells that has very low amount of fodrin, we overexpressed an α-fodrin EGFP plasmid in HEK293 cells. α-fodrin overexpression led to an increase in the level of acetylated tubulin whereas the total tubulin or gamma-tubulin amount remained the same. It has been earlier shown that the down regulation of α-fodrin has effects on the cell cycle, specifically a G1-S arrest. In light of this, cell cycle analysis upon overexpression of α-fodrin in HEK 293 cells revealed that there was a shift towards S phase with simultaneous decrease in the G1 phase. Besides, overexpression of α-fodrin led to a significant increase in the number of cells with mitotic abnormalities in the form of unattached chromosomes. Analysis of the spindle assembly checkpoint proteins revealed that the level of the protein MAD2 had significantly reduced which possibly explained the chromosome detachment. Further, a limited study showed a significant increase of gamma tubulin at the centrosomes when α-fodrin was overexpressed.

Abbreviations: DMEM - Dulbecco's Modified Eagle Medium, γTuRC- Gamma Tubulin

Ring Complex, γTuSC - Gamma Tubulin small Complex, α-fod - alpha fodrin, β-fod - beta fodrin, FBS- Foetal Bovine Serum, BSA - Bovine Serum Albumin, Hek293 - Human Embryonic Kidney 293, MAD2- Mitotic Arrest Deficient protein 2, BUB1 Budding Uninhibited by Benzimidazole protein 1, PVDF Membrane- Polyvinylidene difluoride membrane, PBS - Phosphate Buffer Saline, TBST- Tris Buffer Saline + 0.1% tween20, FACS - Fluorescence Activated Cell Sorter, GAPDH - Glyceraldehyde 3-phosphate dehydrogenase

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OBJECTIVES.

1. To express alpha-fodrin EGFP in HEK 293 cells and check its effect on cell cycle
2. To check the effect of overexpression of alpha fodrin on
   i) microtubule related proteins like tubulin, acetylated tubulin and gamma tubulin
   ii) spindle assembly checkpoint proteins Bub1 and MAD2
3. To study the effect of fodrin overexpression on mitosis and gamma tubulin localization at centrosomes.

INTRODUCTION.

Cell cycle consists of events that take place in particular order throughout the life of a cell that eventually lead to its division into two daughter cells. The phases of cell cycle are G0 or resting phase, interphase which is again divided into G1, S and G2 and finally M phase or mitotic phase (Cooper GM., 2000). Out of these, M phase is where the separation of sister chromatids of the chromosome happens. This process of segregation happens with the aid of spindle fibres which are formed of microtubules.

Cytoskeleton is a structure of immense importance in every living cell as they help in maintaining the structural integrity and normal functioning of the cell. In eukaryotic cell the cytoskeleton is composed of microtubules, microfilaments and intermediate filaments (O'Connor et al., 2010). Microtubules play important roles in various cellular processes such as cell motility, transport, cell division etc. During mitosis, the highly dynamic microtubules help in the movement of chromosomes to the poles of a cell by forming bipolar spindles(Doxsey S., 2001) in eukaryotes. The process also involves the polymerisation – depolymerisation of tubulin: microtubule system (Rochlin et al., 1999). Microtubule is a polymer of alpha (α) and beta (β) tubulin. A microtubule is a hollow cylindrical structure formed from thirteen microtubule protofilaments, with the inner and outer diameters of this is 12 nm and 25 nm respectively (Anna Akhmanova et al., 2008) (Figure 1).

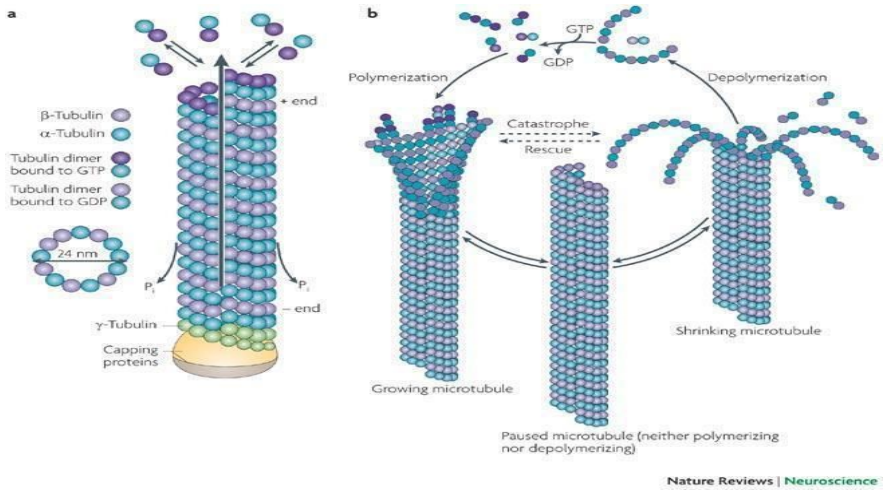


Figure 1: **Dynamics of microtubules** adapted from Anna Akhmanova et al.

Gamma tubulin is a protein which belongs to the tubulin superfamily of protein but is different from alpha and beta tubulin in function (Oakley BR, 1992, Zheng Y et al., 1995). Gamma tubulin is found associated as a complex called gamma tubulin ring complex in the higher eukaryotic organisms (Zheng Y et al., 1995). The gamma tubulin ring complex is composed of gamma tubulin and some other gamma tubulin binding proteins which is recruited to various microtubule organizing centers like centrosomes (Conduit. P. T., 2016). Gamma tubulin ring complex is present both in centrosome and cytoplasm (Gunawardane RN et al., 2000), but the nucleation of microtubules happen only from the centrosome suggesting that there is an inhibitory mechanism working in the cytoplasm that prevents nucleation (Sasidharan Shashikala et al., 2013; Zheng Y et al., 1995). γ-TuRC is always present in the centrosome but prior to the onset of mitosis

its concentration increases rapidly (Sasidharan Shashikala et al., 2013; Khodjakov A et al., 1999). The γ-TuRC forms a helical ring-like structure with a diameter and pitch that closely matches to that of a microtubule (Kollman et al., 2015). Gamma Tubulin Small complex (γ-TuSC) is formed by the combination of two molecules of gamma tubulin with GCP2 (Gamma Tubulin Complex Protein 2) and GCP3. Seven of these combine with GCP 4, 5 and 6 to form Gamma Tubulin Ring Complex (Neus Teixido-Travesa, 2012)(Figure 2). Though γ-TuSC is capable of microtubule nucleation, in the case of higher eukaryotes γ-TuRC acts as the nucleator which has a higher fidelity (Choi YK et al., 2010). The ring complex acts as a template on which rapid addition of tubulin monomers happen, to form microtubules (Alberts B et al.(2002)). There are evidences that suggest that the localization of gamma tubulin ring complex is regulated by proteins like Nedd 1 which is again controlled by phosphorylation through Plk1 and Cdk1 (Xiaoyan et al,, 2009)

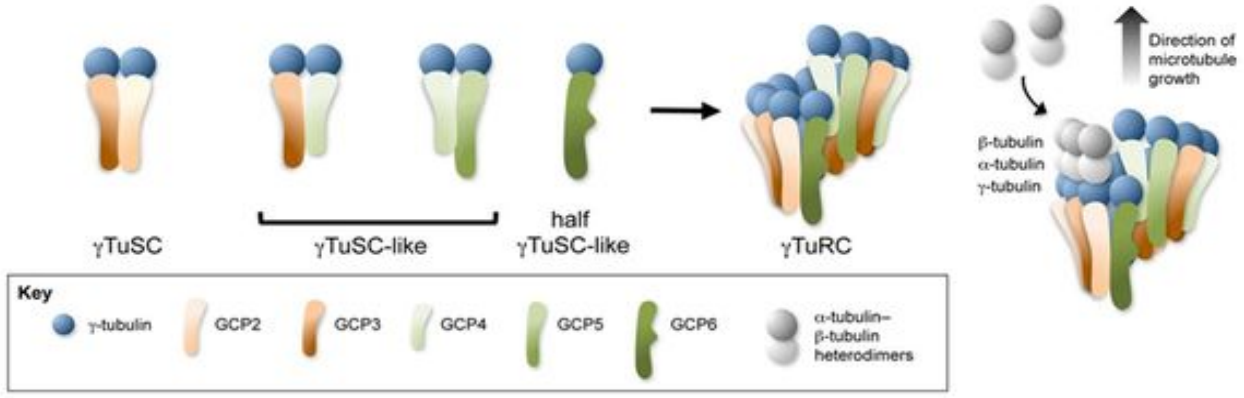


Figure 2: Gamma Tubulin Ring Complex and the nucleation of microtubules. Adapted from Neus Teixido-Travesa et al.

Earlier it was found from this laboratory that fodrin is a part of gamma-tubulin ring complex isolated from goat brain (Nisha et al., 2010). Further it was identified that fodrin is present in the centrosomes of brain specific cell lines in association with gamma tubulin (Sasidharan Shashikala et al, 2013). Fodrin/non-erythroid spectrin is a closely related tetrameric complex composed of two subunits, 240 kDa (α) and 235 kDa (β), which is abundant in brain cells (Riederer BM et al). Spectrin was first discovered in erythrocytes as part of membrane skeleton (Painter. R. G et al., 1975). α subunit of fodrin contains a domain which has a calmodulin binding site (Simonovic M et a.l, 2006) and β subunit have site for membrane attachment that contains a binding site for syndein/ankyrin (Goodman SR et al.,1986)(Figure 3).

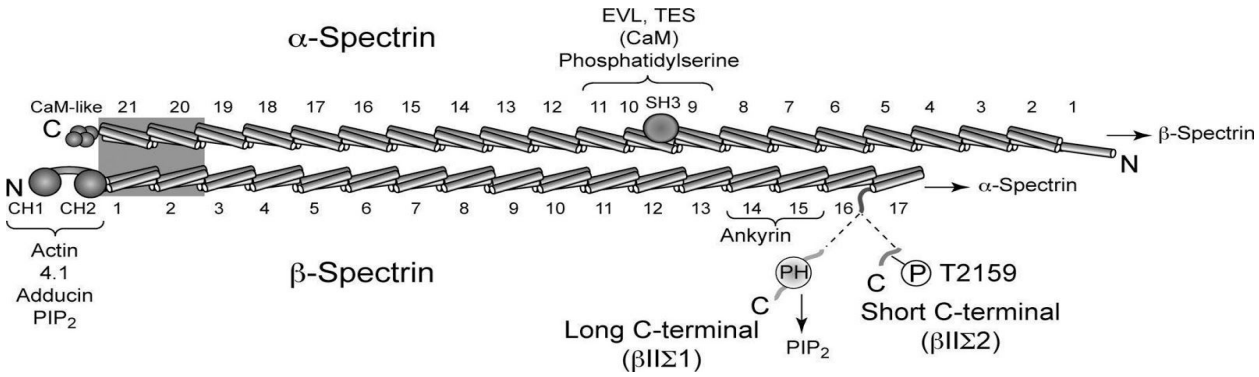


Figure 3: Spectrin tetramer chain - Adapted from Anthony. J. Baines , 2009

Fodrin, a non erythroid spectrin, is a cytoskeleton protein whose functions are mostly associated with providing mechanical strength to the cytoskeletal structure by forming a pentagonal or hexagonal structure and thus supporting the plasma membrane (Gratzer WB et al, 1975). Spectrin also plays roles in vesicle transport by interacting with their early forms along with dynein (Watabe H et al., 2008). Fodrin interact with dynactin

through ARP1 which provides a link to dynein and other motor proteins (Holleran et al., 1996; De Matteis et al., 2000, Sasidharan Shashikala et al., 2013). However, recently some newer functions of fodrin like role in cell cycle regulation, apoptosis and DNA repair mechanism have also been observed (Elmore. S et al., 2007).

In the lab the major focus is on understanding the role of fodrin in microtubule related functions as it was identified as part of the ring complex in brain (Nisha et al, 2010). When α-fodrin was down regulated it was observed that there is reduction in mitotic population with concurrent increase in mitotic abnormalities (unpublished observation). Further there is reduced localization of the gamma tubulin at the centrosomes upon alpha fodrin depletion (unpublished data). To better understand the functions, we undertook the study to determine the effect of alpha fodrin. Since there was difficulty to transfect a large plasmid into brain specific cell lines, we focussed on studying overexpression in cell lines which apparently have reduced level of fodrin. Thus the current study focusses on understanding the role of fodrin overexpression on cell cycle, microtubule organization and gamma tubulin localization at the centrosome. We have observed in this current study that overexpression of fodrin leads to changes in cell cycle phases, aberrant mitotic cells and changes in the levels of spindle assembly checkpoint protein MAD2.

MATERIALS.

DMEM(Gibco), FBS(PAN Biotech), T25 flasks (nunc), 35mm Petri dishes(nunc), Lipofectamine 2000 (ThermoFisher SCIENTIFIC), Opti MEM (Invitrogen), Fluoromount-G (Electron Microscopy Sciences),10 µg/ml Hoechst 33342 (ThermoFisher SCIENTIFIC). All the other reagents were of reagent grade obtained basically from Sigma Aldrich.

HEK293 cells were obtained from ATCC.

Primary antibodies: Mouse anti Gamma Tubulin(1:1000, Pierce), Rabbit anti GFP(1:400, CST), Mouse anti tubulin(1:800, CST), Rabbit anti Mad2 (1:400, abcam). Rabbit anti fodrin(1:1000), mouse anti GAPDH (1:5000, Pierce), rabbit anti Bub1(1:1000, Abcam). Ms anti acetylated tubulin(1:1000, Santacruz)

Secondary antibodies: Anti mouse alexa 568 (1:800), anti rabbit alexa 488 (1:400), anti mouse alexa488 (1:600), anti rabbit 568 (1:600) were obtained from invitrogen. Anti mouse HRP(1:1000), anti rabbit HRP(1: 1000), anti mouse HRP(1:1000) were obtained from Sigma- Aldrich.

METHODS

Overexpression of alpha fodrin in HEK293 cells.

1. HEK 293 cells were cultured in DMEM with 5% foetal bovine serum in a T25 flask as a monolayer.
2. α-fodrin EGFP plasmid was already generated in the lab by cloning α-fodrin into EGFP-C1 vector. For over expression and cell cycle analysis, HEK 293 cells are seeded in 35mm dishes. EGFP (control) or α-fodrin EGFP was transfected with Lipofectamine 2000 and cells were collected after 48hrs.
3. Transfection: the concentration of plasmids EGFP-C1(control) and α-fodrin EGFP was calculated using uv-visible spectrophotometer(concentration of EGFP-C1-1124ng/μL, concentration of α-fodrin EGFP- 1106.25ng/μL). The volume of this mixture that was added to one 35mm petridish is 200μL. 3 μg plasmid+7.5μL lipofectamine 2000 (a cationic liposome) was added to 189.5μL OptiMEM. This is incubated at 37°C for 15 minutes. Meanwhile the media (DMEM) in the petridishes containing cells have been changed. To this the mixture (plasmid+lipofectamine 2000+OptiMEM) was added.

Western blot.

The cells were collected with DMEM from 35 mm dish by scraping while the petri dish containing cells was kept on ice. This is pelleted by centrifugation at 4000 rpm for 7 minutes AT 4ºC. The scraping is repeated after adding 1X PBS to the petri dish. This too is pelleted by centrifugation. The pellet that has been obtained finally is washed with 1X PBS. After discarding the PBS, cell pellets were treated with phospho lysis buffer containing protease inhibitors. The cell pellets are incubated for 45 minutes vortexing it

once in every 15 minutes. This is again kept for centrifugation at 13000 rpm for 5 minutes at 4ºC. Estimation of protein is done using Bradford's solution. Gel loading samples were prepared by adding 5X protein loading dye. The prepared cell lysates were run in 8%,10%, 12% SDS PAGE as required, transferred onto a PVDF membrane, blocked using 5% milk in 1X TBS-T and probed using respective primary and secondary antibodies prepared in 3%BSA with appropriate dilutions. The blot was developed using Enhanced chemiluminescence kits (Pierce ECL Western blotting substrate from Thermo Fisher SCIENTIFIC) followed by exposing it into an X-ray film. The bands of protein were analysed and levels were quantified using densitometry analysis softwares (Bio Rad).

Cell cycle analysis.

The HEK293 cells were transfected with EGFP or α-fod EGFP and cells were pelleted after 48hrs, washed with media and incubated with 10 µg/ml Hoechst 33342 for 30 minutes at 37°C.They were then strained through a 40µm nylon cell strainer to get single cell suspension. The cells were then analysed in FACS-Aria 2 cell cycle analyser using approximately 10000 cells for each analysis. BD FACS-Diva software was used for the data analysis.

Immunofluorescence assay

The cells were grown in coverslips and transfected using EGFP and α-fod EGFP and incubated for for 48 hours. After removing DMEM from the petri dish the cells were washed using 1X PBS kept at 37ºC. The cells were then fixed using chilled methanol for 10 minutes at - 20ºC. This is then washed with 1X PBS. Blocking is done using 5%BSA in 1X PBS containing 0.1% Triton X 100 at 37ºC for 30 minutes. They are then

incubated with primary antibody (prepared in 5% BSA in 1X PBS containing 0.1% Triton X 100) overnight at 4°C. After washing 3 times with 1X PBS for 5 min, secondary antibody was added and kept at room temperature for 2 hours. Washing is repeated and DAPI was added following this and mounted on a slide using fluoromount-G.

ROI calculation

HEK293 cells transfected with EGFP or alpha fodrin EGFP plasmids were fixed and immunocytochemistry was performed using gamma tubulin and GFP antibody. Localization of gamma tubulin was detected by Z stacking, background corrected and maximum intensity profiles were generated using Nikon's NIS Elements software. A circular ROI (region of interest) was drawn around the centrosome and mean intensity over that area was determined. For our analysis the total intensity was calculated as a product of mean intensity and ROI area.

Statistical Analysis

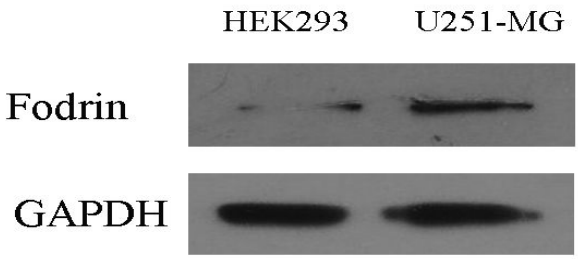
For western blots, densitometry was conducted using BD Quantity One software (Bio-Rad). Data was normalised to loading control before statistical analysis. Expression of HEK293 EGFP transfected cells was taken as one and fold change was calculated for HEK 293 alpha fodrin GFP cells. Results were depicted as mean ± standard deviation. p-values were estimated using Student's unpaired *t*-test. Value of $p < 0.05$ was considered to be statistically significant.

RESULTS.

To express Alpha fodrin EGFP in HEK 293

The lab had already constructed an alpha fodrin EGFP plasmid (12kB). Since it was difficult to transfect this plasmid into brain specific cell lines, we selected HEK293 cells for our overexpression studies as it had low intrinsic levels of fodrin (Figure 4 A) and the transfection was relatively easy.

A



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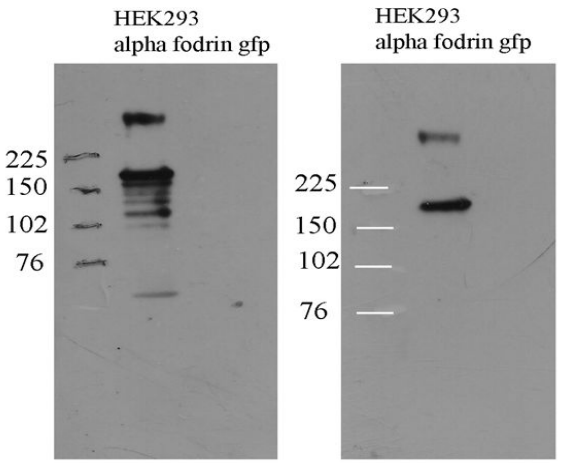


Developed with GFP antibody Developed with fodrin antibody

C

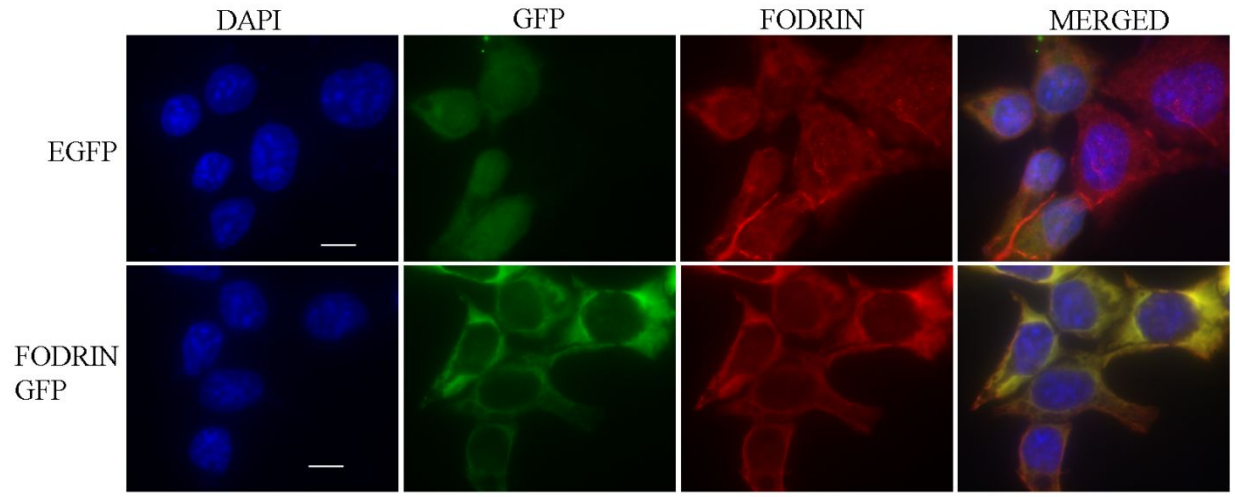


Figure 4: Expression of alpha fodrin in HEK 293 cells.

A- Western blot showing the intrinsic levels of alpha fodrin in HEK 293 and U251MG cells.

B- α fodrin EGFP in HEK 293 were developed using GFP antibody (left) and fodrin antibody (right) respectively.

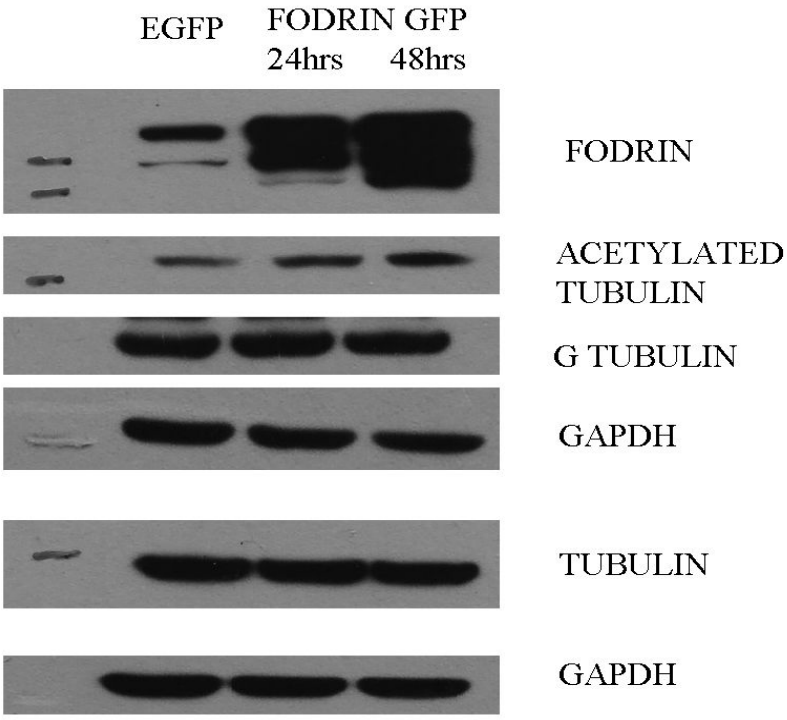
C- Verification of over expression of functional alpha fodrin in HEK 293 cells using immunofluorescence assay . Top panel shows HEK 293 cells transfected with EGFP and the bottom panel shows cells transfected with α-fodrin EGFP. Image was taken in oil immersion, 60X objective using Olympus IX 71 Fluorescence microscope. Scale bar represents 5μmeter.

Upon alpha fodrin overexpression in HEK293, the protein was verified by western blot using both GFP and fodrin antibody (Figure 4B). Further, immunofluorescence experiment showed the recognition of the overexpressed fodrin with fodrin antibody indicating it to be a functional protein (Figure 4C).

Effect of α-fodrin overexpression on microtubule related proteins

HEK 293 cells were transfected using EGFP (control) or α-fodrin EGFP (over expression). To study the effect of overexpression on microtubule related proteins, samples were collected at 24 and 48 hrs post transfection, lysed and western blotting was performed. The blot was then developed for tubulin, acetylated tubulin and gamma tubulin, with GAPDH as the loading control (Figure 5A). The levels of tubulin and gamma tubulin were similar in control and alpha fodrin overexpressed samples. However, the level of acetylated tubulin increased when α-fodrin was overexpressed (Figure 5B). This increase was more than 50% after 24 hours of transfection.

A



B.

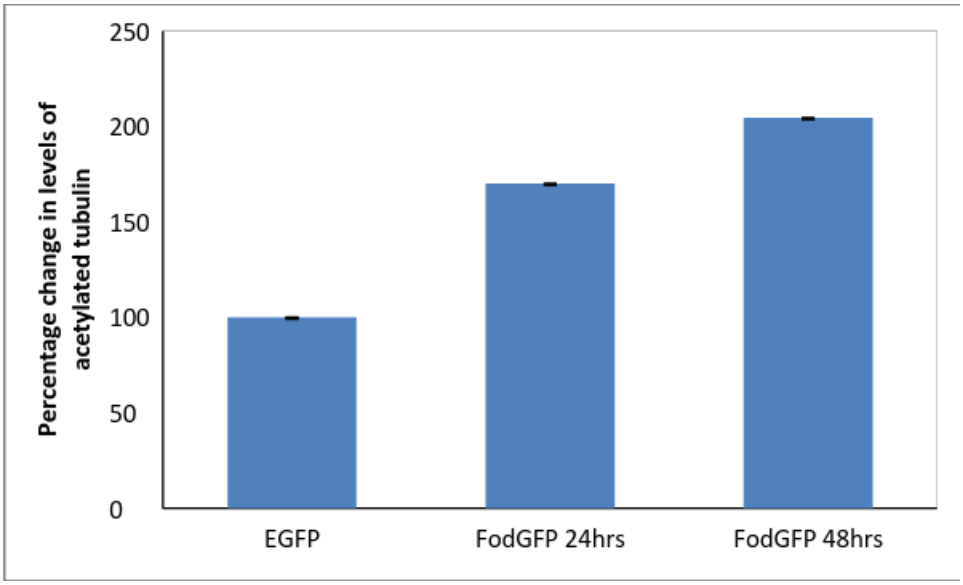


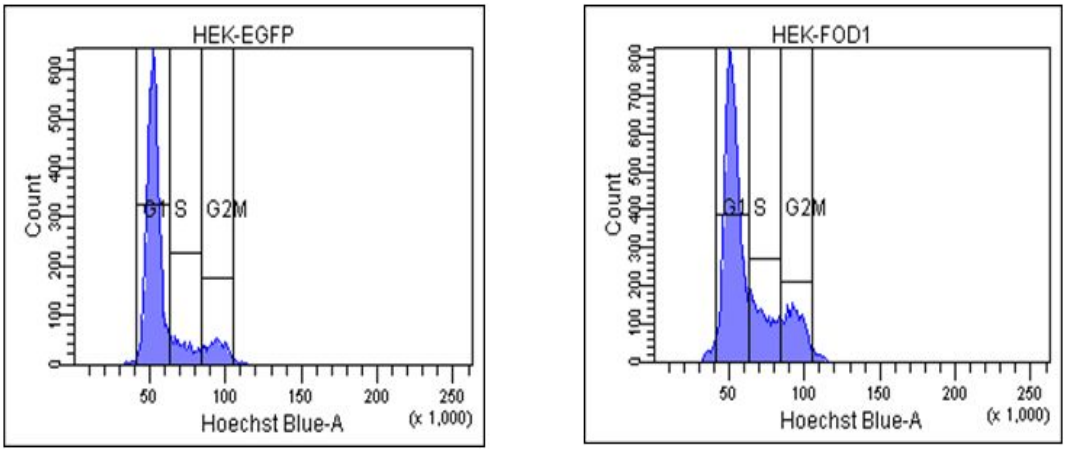
Figure 5: Effect of overexpression of α-fodrin on microtubule related proteins in HEK293 cells. A. Western blot analysis of effect of overexpression of α-fodrin. The top most band shows the over expression of α-fodrin. B. Quantification of the western blot showing an increase in the level of acetylated tubulin. The quantification was done twice.

Effects of α-fodrin overexpression on cell cycle.

To understand the effect of fodrin overexpression on cell cycle, HEK293 cells were transfected with either EGFP or α-fodrin EGFP plasmid and incubated for 48hrs at 37°C. The cells were then collected and incubated with Hoechst 33342. The dye stains the DNA and depending upon the cell phases the amount of dye binding changes. Thus the amount of dye binding will be in the order of G2/M>S>G1. The quantification can then be analysed using FACS. The GFP positive cells were gated from the above cell

population and the positive cells were analysed for their cell cycle phases (Figure 6A).

A



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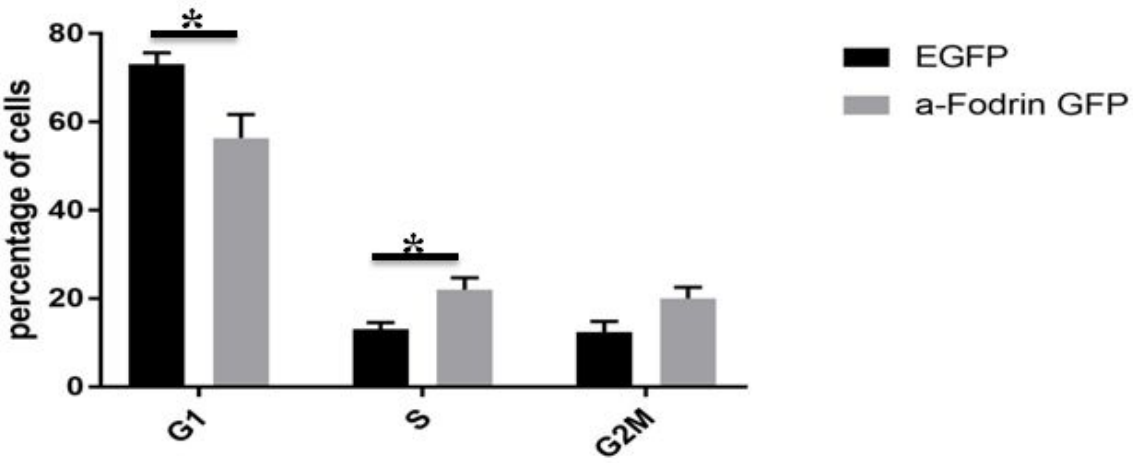


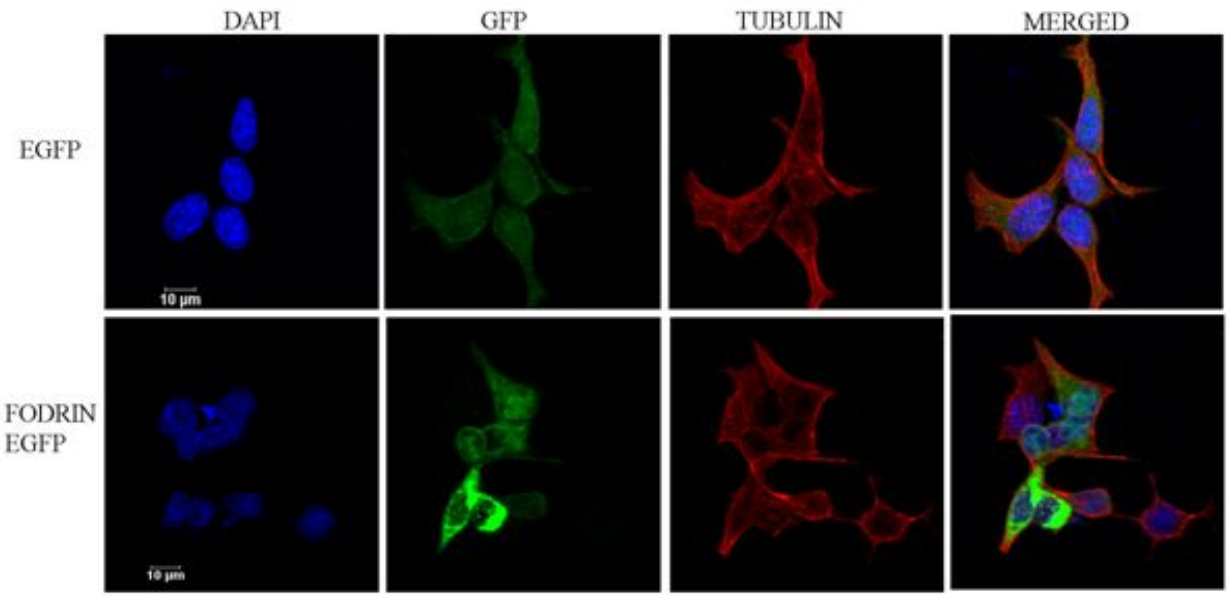
Figure 6:Analysis of cell cycle after α-fodrin overexpression in HEK 293 cells using FACS.
A. Image showing the number of cells in G1, S and G2/M phase from EGFP and α-fodrin-GFP expressing cells respectively.
B. Quantification of the percentage of cells from figure A. A decrease in the percentage of cells in G1 phase coupled with increase in percentage of cells in S and G2/M phase was observed. Increase in the S phase was significant. This experiment was performed four times. * represents p value. p value for G1 is 0.0153, for S phase is 0.0231.

On analysis it was observed that upon fodrin overexpression, there was a decrease in G1 phase coupled with a significant increase in S phase (Figure 6, B). This indicates that fodrin is involved in regulation of cell cycle by causing a transition from G1 to S phase. It should be noted here that fodrin downregulation causes G1 arrest (unpublished data).

Effect of α-fodrin on microtubule organization.

Immunofluorescence staining was performed for cells transfected with EGFP or Fodrin-EGFP plasmids and then stained for tubulin and GFP. Z stack images were then obtained using confocal microscope and cells were categorized as interphase and mitotic cells. There was no apparent change in interphase cells (Figure 7A) but there was observation of mitotic abnormalities upon alpha fodrin overexpression (Figure 7B). While EGFP transfected cells had normal mitosis (not counted but visibly observed), fodrin-EGFP transfected cells had unattached chromosomes as shown in the figure (Figure 7B).

A


DAPI
GFP
TUBULIN
MERGED
EGFP
FODRIN
EGFP
10 µm
10 µm

B

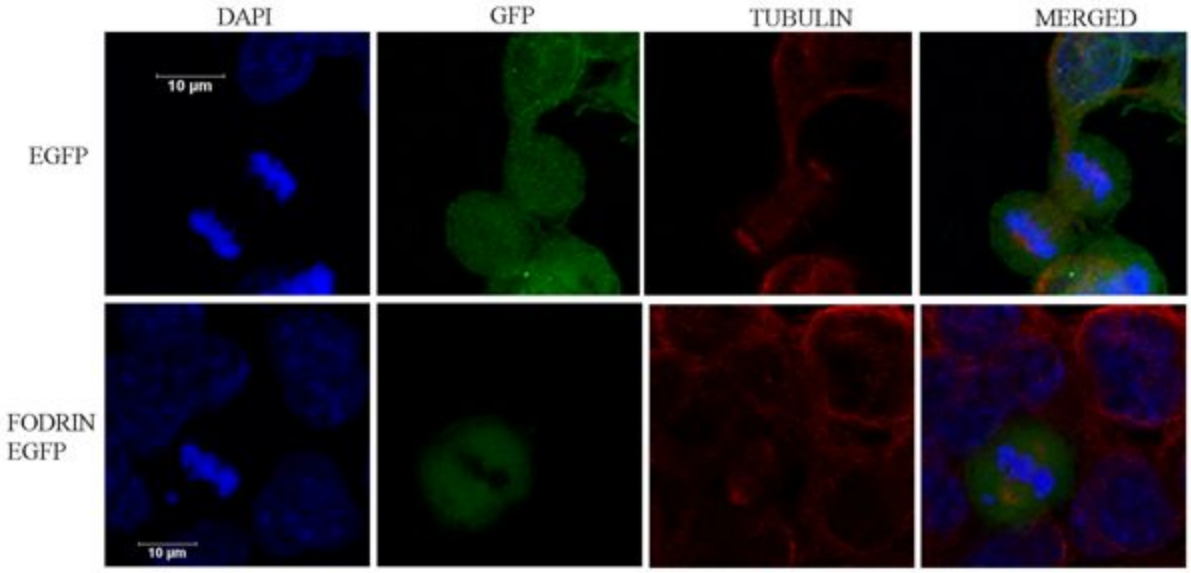
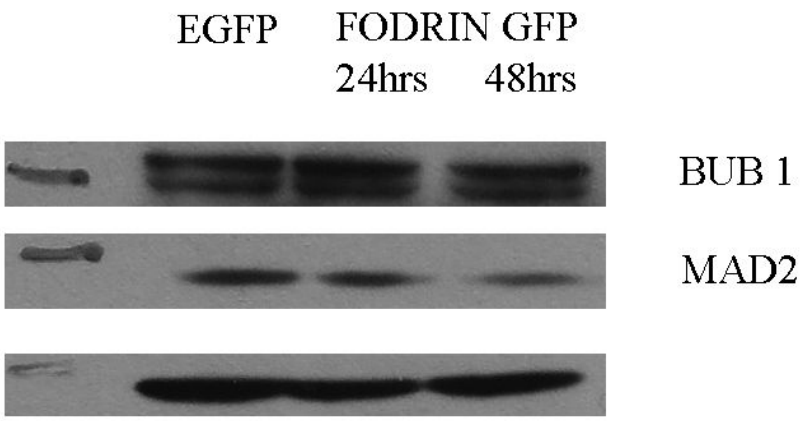

DAPI
GFP
TUBULIN
MERGED
EGFP
FODRIN
EGFP
10 µm
10 µm

Figure 7: Figures showing the effect of α-fodrin on microtubule organization in HEK 293 cells.
A- Interphase cells showing normal mitotic spindles.
B- Mitotic errors in the form of unattached chromosomes when fodrin was overexpressed. The panel of DAPI staining in image B shows this.

Effect of α-fodrin overexpression on spindle assembly checkpoint proteins

Since we observed mitotic abnormalities upon fodrin overexpression, western blot analysis of the checkpoint proteins Bub1, MAD2 was performed. There was no change in total protein level of Bub1 but there was a decrease in the level of MAD2 upon fodrin overexpression (Figure 8 A and B).

A



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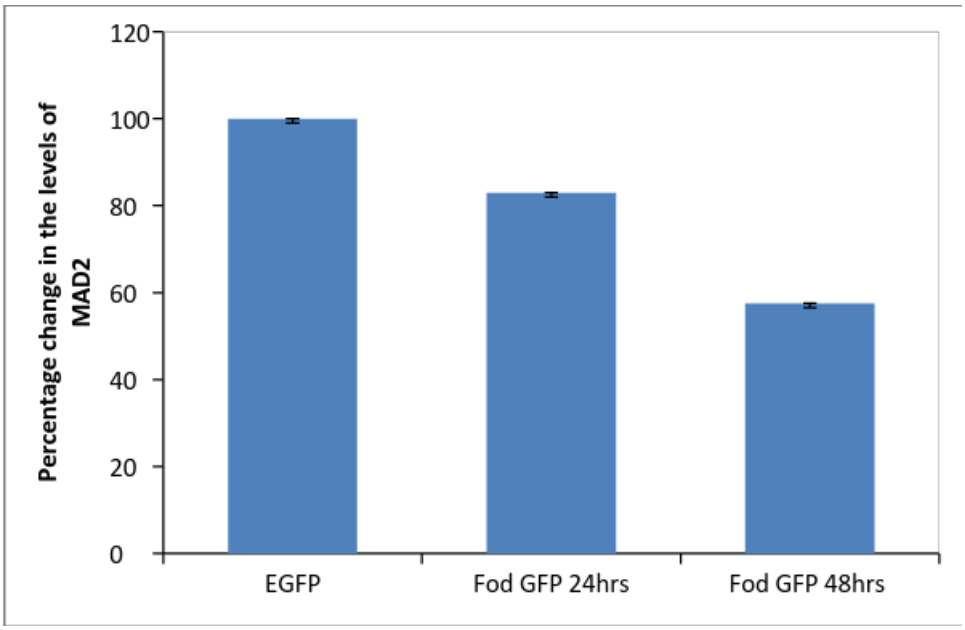

Figure 8: Effect of overexpression of α-fodrin on spindle assembly checkpoint proteins in HEK 293 cells.

A. Western blot analysis of the levels of BUB1 and MAD2.

B. Quantification of the western blot results for the level of MAD2 which decreased upon overexpression of α-fodrin. The experiment was done twice.

Further, immunofluorescence assay was done for cells transfected with EGFP or α-fodrin EGFP plasmids in which MAD2 and GFP were stained. Z stack images were then obtained using confocal microscope. Similar to western blot results there was a decrease in the level of MAD2 upon fodrin overexpression.

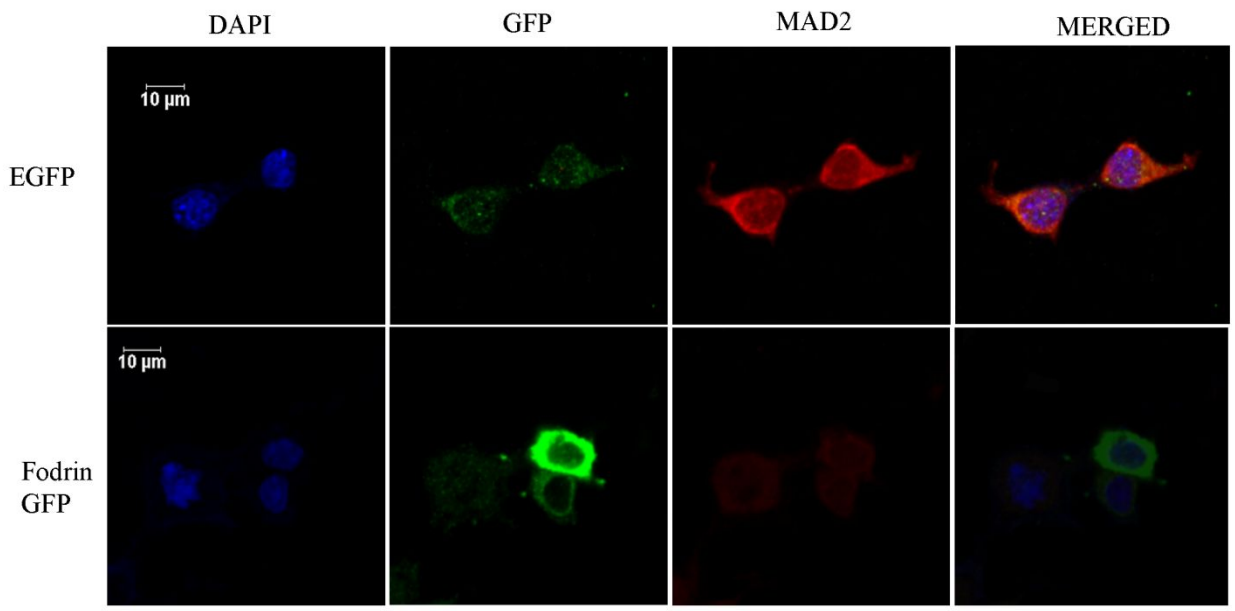

Figure 9: Immunofluorescence Assay showing changes in MAD2 level upon α-Fodrin overexpression in HEK 293 cells.
The intensity of the green channel was changed as required in the instrument while the red channel intensity was not changed. The bottom panel showed that the intensity of MAD2 decreased when α-fodrin was overexpressed.
This experiment was done once.

Effect of alpha fodrin on gamma tubulin localization

Further, to check what happens of the gamma-tubulin level upon fodrin overexpression, an experiment was done for cells transfected with EGFP or α-fodrin EGFP plasmids. Cells were stained for gamma tubulin and GFP and Z stack images were obtained using confocal microscope with the intensity of the gamma tubulin channel being untouched. An ROI area was drawn around the centrosome stained by gamma tubulin and the total intensity of gamma tubulin was calculated and analysed by the formula:

Gamma tubulin intensity= Mean intensity*ROI area

The level of gamma-tubulin increased after alpha fodrin overexpression as shown in Figure 10 and 11.

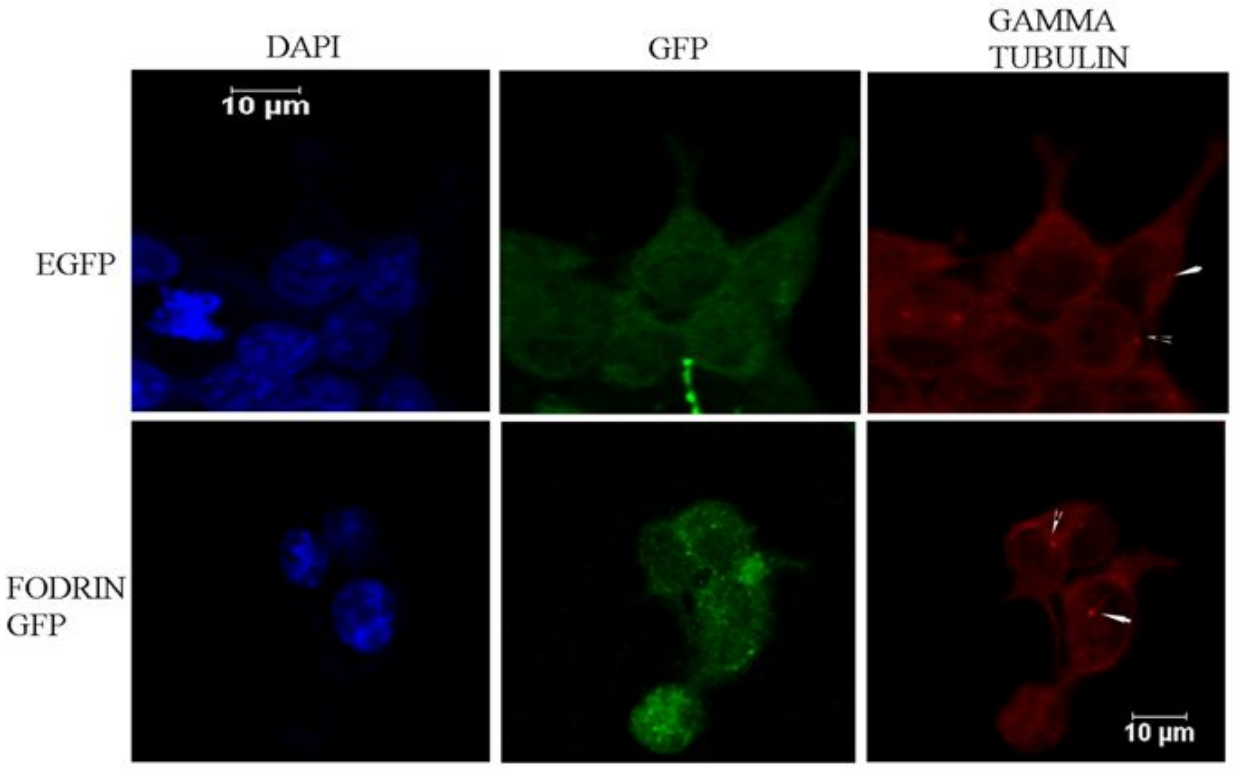

Figure 10: Immunofluorescence assay of Effect of alpha fodrin on gamma tubulin localization in HEK293 cells. Arrow marks indicate the localisation of gamma tubulin at the centrosome. The intensity of gamma tubulin(red) at centrosome is increased in alpha fodrin overexpressed cells.

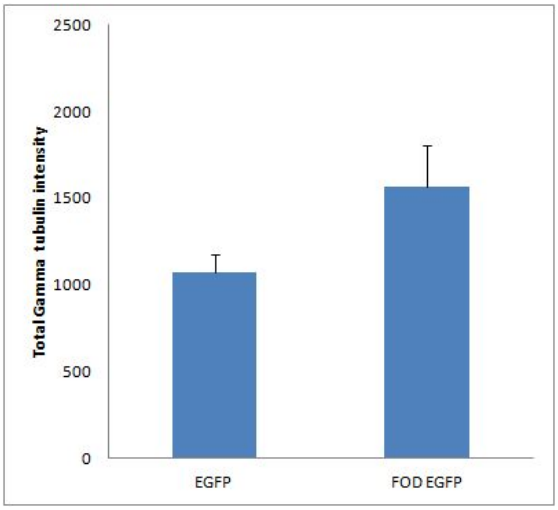

Figure 11: Quantification of gamma tubulin localization for both normal and α-fodrin GFP transfected cells.

The localization of gamma tubulin at centrosome increases when α-fodrin was over expressed.

This experiment was done once.

DISCUSSION

Earlier experiments from the laboratory using fodrin shRNA indicate that α-fodrin is important in the transport of gamma-tubulin from the cytoplasm to the centrosome in brain derived cell lines. With this in mind, we have tried to overexpress α-fodrin in cells. However, since overexpression of α-fodrin, a very big protein, was so far not possible in neuroblastoma or glioblastoma cells, we have transfected HEK293 cells with cloned α-fodrin E-GFP plasmid, which originally has very little amount of fodrin. Overexpression of α-fodrin kept the label of tubulin and gamma-tubulin similar, but the amount of acetylated tubulin had increased. Acetylated tubulin is a variant of tubulin which normally shows an increase in the stability of tubulin (Cueva et al, 2012). Thus the increase in the level of acetylated tubulin upon the increase in alpha fodrin indicates that fodrin is involved in maintaining the stability and dynamics of microtubules.

It has been observed previously from this lab that α-fodrin downregulation resulted in an arrest in the G1 phase of cell cycle (unpublished data). Analysis of cell cycle upon α-fodrin overexpression showed that there was a significant reduction in the number of cells in G1 phase coupled with an increased number of cells in S phase (figure 6). The data hence indicate that fodrin somehow helps in the transition of cells from G1 to S phase of cell cycle in normal HEK 293 cells.

Further fodrin overexpression has led to abnormal mitosis in the form of unattached chromosomes. MAD2 is a spindle assembly checkpoint protein (J.V. Shah

et al., 2000; Li Y, Benezra R et al., 1996). Normally in a cell the function of this is to check whether every chromosome is attached to the bipolar spindle prior to mitosis. In the normal level of MAD2 if there is an unattached chromosome the cell will not proceed to mitosis (J.V. Shah et al., 2000). So when the level of MAD2 is less the cell will undergo division even if the chromosomes are not properly attached to the bipolar spindle. This might lead to the transfer of mitotic errors to the next generation. Thus our observation of a reduction in the level of MAD2 could allow the cells to have unattached chromosomes but still facilitate the cells to complete cell division. This indicates that fodrin may play a major role in maintaining the genomic stability via spindle assembly checkpoint proteins.

In our limited study we have also observed that the localization of gamma tubulin at the centrosome increases in presence of transfected fodrin in α-fodrin EGFP cells. Gamma tubulin itself has been identified as a marker of centrosome. Hence this indicates that α-fodrin is involved in the localization of gamma tubulin to the centrosome. This observation is complementary to our earlier observation that the amount of gamma-tubulin decreased in the centrosome upon fodrin downregulation in brain derived cells (unpublished observation).

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