THE SAHLGRENSKA ACADEMY

Regulation of ASK3 inactivation under hyperosmotic stress

Degree Project in Medicine

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1. Abstract

Background: Hypertension today is common and a great risk factor for cardiovascular disease (CVD). WHO states that more people die annually from CVDs than from any other cause. This project focuses on the regulation of apoptosis signal regulating kinase 3 also known as ASK3, which has been observed to regulate blood pressure in the kidney. To clarify the regulatory mechanism of ASK3 activity is important for the development of new drugs against hypertension. It has been suggested that transient receptor potential cation channel, mucolipin (TRPML1), a non selective cation channel, might be the upstream regulator of ASK3.

Objectives: To investigate whether TRPML1 is necessary for the regulation of ASK3 under hyperosmotic stress through different laboratory techniques.

Methods: The research was conducted at The University of Tokyo, laboratory of cell signaling during September to November 2016. To answer the research question many different laboratory technics were used. Such as immunoblotting, immunostaining, immunoflourecense and cell culturing.

Results: One of the five experiments supported the hypothesis that TRPML1 would be the upstream regulator of ASK3 under hyper osmotic stress. Another experiments gave negative results, thus one could not draw the conclusion that TRPML1 is a regulator of ASK3. A TRPML1 KO cell line was also established for further experiment in this field.

Conclusion: Three months is not enough to draw any conclusions regarding the regulation of ASK3 but the results showed some promise. The experiments needs to be repeated and re-evaluated. The experiment that gave negative results could be due to my short experience in the lab, so if done properly again it might suport the hypothesis. Thus, it is important to continue this research since a regulatory mechanism of ASK3 is identified, a new therapeutic strategy for hypertension may be developed and help many people around the world.

2. Background

The animal cell is surrounded by a plasma membrane. One of the cell membranes many properties, is that it is highly permeable to water but at the same time impermeable to the macromolecules inside the cell. The water is able to move through the cell membrane by the lipid bilayer and aquaporins (AQPs), which are water selective channels [1]. It is because of these properties that the cells are always in danger of water influx and swelling, which without control mechanisms, will lead to cell lysis. This swelling and lysis can be avoided because the cells are able to regulate ion distribution over the plasma membrane [2]. Due to changes in the extracellular and intracellular concentration, an osmotic gradient will arise over the plasma membrane, this will result in water influx/efflux and a change in cell volume. These changes affects the cells function and is know as osmotic stress. Fortunately the cell is equipped with countermeasures against cell swelling/cell shrinking and can quickly restore its normal volume. There are many signaling pathways in the cell that controls the cell volume regulation [3,4].

As mentioned above it is important for the cell, after for example osmotic stress, to restore to its normal volume. Therefore in most cells, osmotic shrinkage or swelling is followed by regulatory volume increase (RVI) or regulatory volume decrease (RVD) [5]. These are cell-intrinsic regularity processes which the cells utilize to restore initial cell volume.

Apoptosis signal regulating kinas 3 also known as ASK3, is a member of the MAP3K family. It has been shown that ASK3 is predominantly expressed in the kidney [6]. AKS3 also responds bidirectionally to osmotic stress, it is activated by hypo-osmotic stress and inactivated by hyper-osmotic stress [6]. ASK3 also regulates blood pressure by suppressing WNK1-SPARK/OSR1 signaling in the kidney [6]. ASK3 is therefore expected to play an important role in osmotic stress response.

This project focuses on regulation of ASK3 inactivation by hyper-osmotic stress. It has been shown that failure of ASK3 inactivation under hyper-osmotic stress leads to inhibited RVI and also cell death. Also that ASK3 depletion suppressed RVD after hypo-osmotic stress. This points to that ASK3 is a converter from osmotic stress signal to bidirectional response and that it is important to both RVD and RVI. The results above have been obtained by Dr. Watanabe and Dr. Niwa and are preliminary data, not yet published.

Another thing that is important is that a hypertonicity-induced cation channel (HICC) [7] is reported to be required for RVI. The molecular identity of this channel is still unknown. It has been shown that if HICC is inhibited, inactivation of ASK3 under hyper-osmotic stress is inhibited. It is possible that revealing how ASK3 is regulated under hyper-osmotic stress could lead to identify HICC.

Through genome-wide siRNA screening, preformed by Dr. Watanabe, it was reported that TRPML1, a non-selective cation channel might be the upstream regulator of ASK3. Dr. Watanabe also had preliminary data that shows when TRPML1 was knocked down, ASK3 inactivation was inhibited.

TRPML1 (transient receptor potential mucolipin) is also known as MCOLN1 and is a non-specific cation channel which is predominantly localized on the membranes of late endosomes and lysosomes in all mammalian cell types [8]. Its activity is vital for proper functioning of these compartments. TRPML1 is a six transmembrane domain protein. TRPML1 is also related to mucolipidosis type IV which is an autosomal recessive lysosomal storage disorder [9]. Individuals with the disorder have many symptoms, including delayed psychomotor development and various ocular aberrations. The disorder is caused by mutations in TRPML1 gene.

As mentioned above ASK3 is able to regulate blood pressure [5] and to clarify the regulatory mechanism of ASK3 activity is important for the development of a new therapeutic strategy for hypertension. This is crucial because hypertension is a risk factor for cardiovascular disease (CVD) and according to WHO more people die annually from CVDs than from any other cause [10].

3. Research questions

To investigate if TRPML1 is necessary for the regulation of ASK3 under hyperosmotic stress through different laboratory techniques.

4. Material and Methods

Cell culture and osmotic stress treatment:

HEK293A (human kidney epithelial cells) and Cas9-HEK293A#57 cells were used for the experiments. HEK293A were cultured in Dulbeccos modified eagles medium with high glucose, supplemented with 10% fetal bovine serum (FBS) and 1000U penicillin (PG) 5 ml at 37ºC in a 5% CO2 humidified incubator. Cas9-HEK293A#57 cells were cultured in Dulbeccos modified eagles medium, supplemented with 10% fetal bovine serum, 250µl zeocin and 125µl blastocidin at 37ºC in a 5% CO2 humidified incubator. The cells were exposed to osmotic stress by changing the extracellular solutions. The solutions had two different osmolality, hypertonic 405mOsm and isotonic 308mOsm and the cells were exposed for 10 min.

Immunoblotting:

Extracts from cells were resolved in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto polyvinylidene difluoride membranes. First the membranes

were blocked by 5% skim milk in tris-buffered saline containing 0.1% Tween (TBS-T), then the membranes were probed with the appropriate antibodies. The antibody-antigen complexes were detected using chemiluminescent western blotting.

Immunostaining:

HEK293A cells were transfected with HA-hASK3, HA-TRPML1 and TRPML1-HA and exposed to hyper- or iso osmotic stress for 10 min. After stimulation, cells were fixed in 4% formaldehyde, permeabilized in 1% Triton X-100. Blocking was performed with 5% skim milk in TBS-T for 30 min, and then incubated with first antibody 3F10 (binding to HA-tag) overnight. Then, cells were incubated with secondary antibody (Alexa Fluor 488 anti-rat IgG). Cells were counterstained with Hoechst after removal of second antibody for 5 min. Images were collected by a confocal microscope.

Immunofluorescence:

Transfected cells grown in a 12 well plate were phospho buffer saline- (PBS)-washed and trypsinised to a 96-well plate, then exposed to hyper- or iso-osmotic stress for 10 min.. After stimulation, cells were fixed in 4% formaldehyde, permeabilized in 0,2% Triton X-100. Blocking was performed with 5% skim milk in TBS-T for 30 min, and then incubated with first antibody 3F10 and 2T over night. Then second antibody was added (Alexa 488 anti-rat and Alexa 594 anti-mouse) Cells were counterstained with Hoechst after removal of second antibody for 5 min. Images were collected by cell-insight. Cell-insight is a high content screening platform and is a system used to measure the light that is being admitted from the cells during immunofluorescence and take pictures.

5. Ethics

No animal experiments were performed and no human materials were used. The Cartagena protocol on biosafety was followed, since genetically modified organisms were created in this project. The protocol protects biological diversity from potential risks posed by genetically modified organisms. It also contributes to ensuring an adequate level of protection as for the safe transfer, handling and use of these modified organisms.

6. Results

Numerous experiments was performed and they are presented in order below.

6.1 Experiment nr 1: Expression vector for TRPML1

At first the expression vector for TRPML1 was created and the experiment was successful. The reason for this experiment was to be able to over express TRPML1 in cells. This is important to determine the sufficiency for TRPML1 regarding the regulation of ASK3. It could also be used in future experiments in the lab. After creation of the expression vector it was transfected into HEK cells, lysed, and SDS-PAGE was performed followed by western-blot detected with antibody. As seen in Fig 1, TRPML1 is over-expressed in a plasmid amount-dependent manner. The middle band is monomeric TRMPL1 because its molecular weight is around 65 kDa and the upper band would be the oligomers. This band patterns corresponds to previous reports.

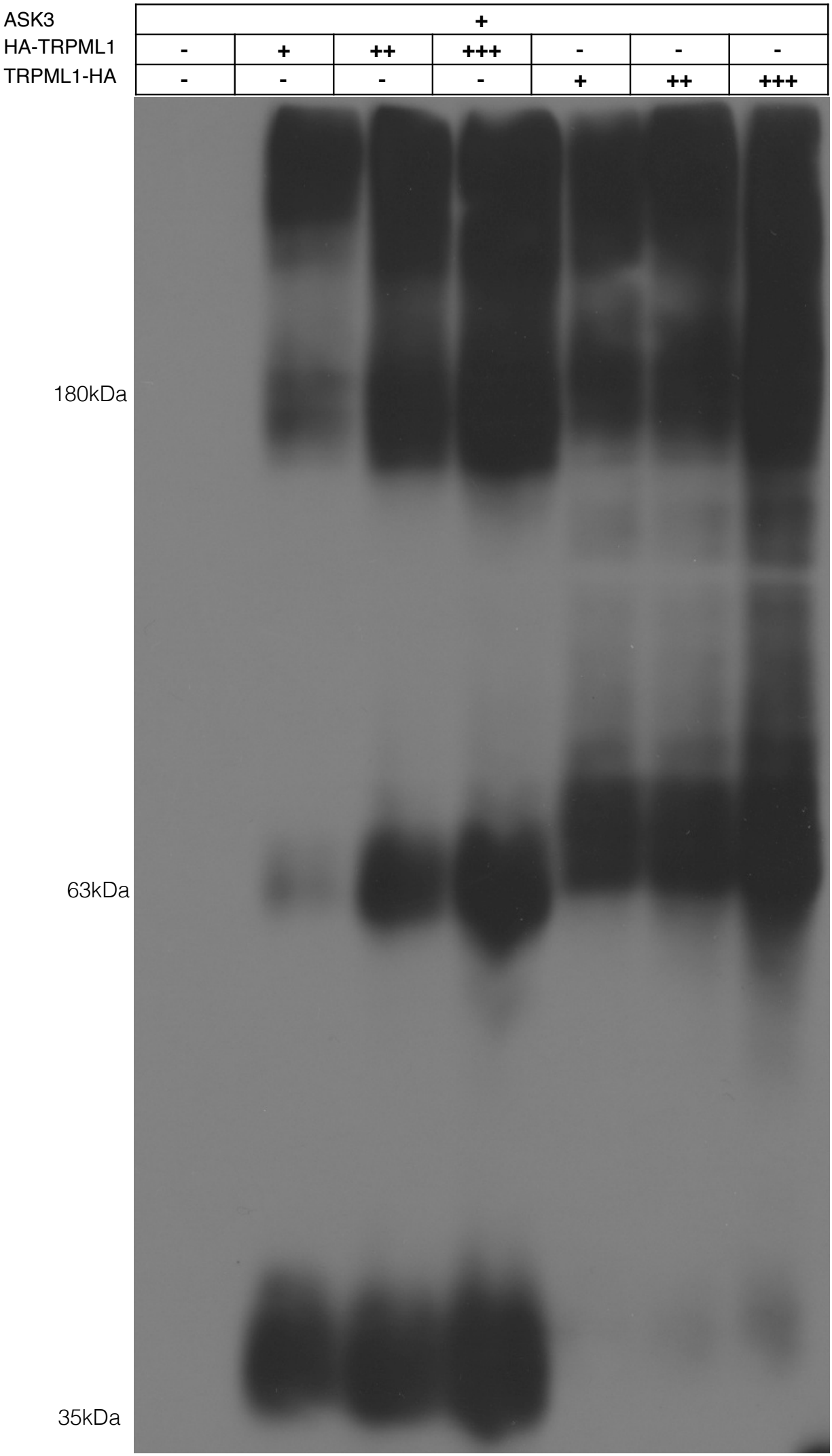


Fig 1, Shows TRPML1 over expression in plasmid amount-dependent manner. HA-TRPML1 and TRPML1-HA is over expressed with increased concentrations from left to right in HEK293A cells. Al lanes was transfected with the same amount of ASK3.

6.2 Experiment nr 2: TRPML1 localization

This next experiment was to examine whether over-expression of TRPML1 caused it to aggregate. Aggregation of TRPML1 would mean lower functionality and misguided results. To exclude the possibility that TRPML1 is over-expressed as an aggregated form, the sub-cellular localization of TRPML1 was examined by immunofluorescence under both hyper- and isotonic stress. Both HA-TRPML1 and TRPML1-HA were used because they will both be included in a later experiment. We included also ASK3 in this experiment to confirm localization that was already known in the laboratory.

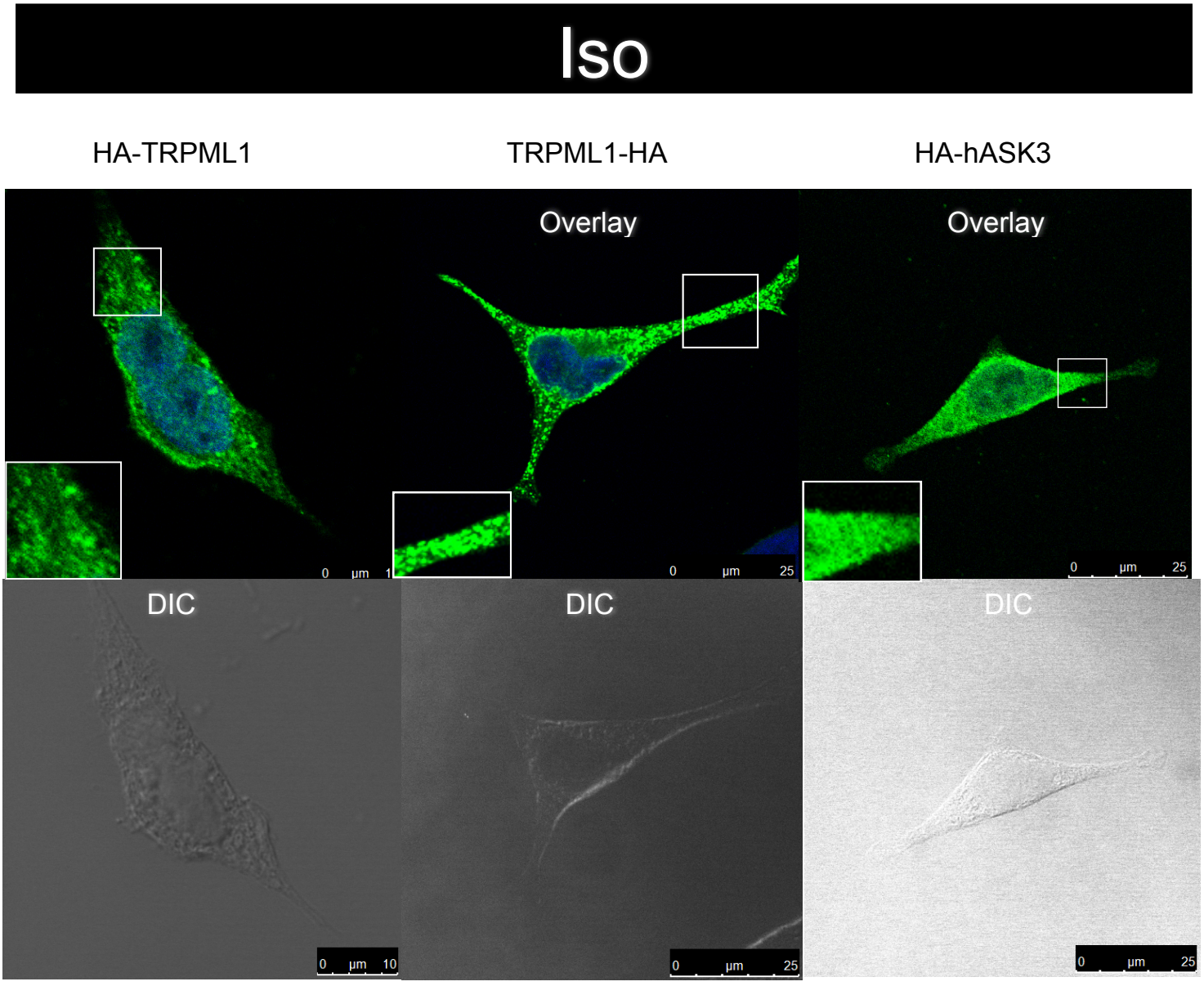


Fig 2, This is localization HA-TRPML1, TRPML1-HA and HA-hASK3 detected by immunofluorescence after 10 min under isotonic stress. DIC is included to show the real position of the cell and that it is confluent with the immunofluorescence pictures. (HA: green, Hoechst staining DNA: blue)

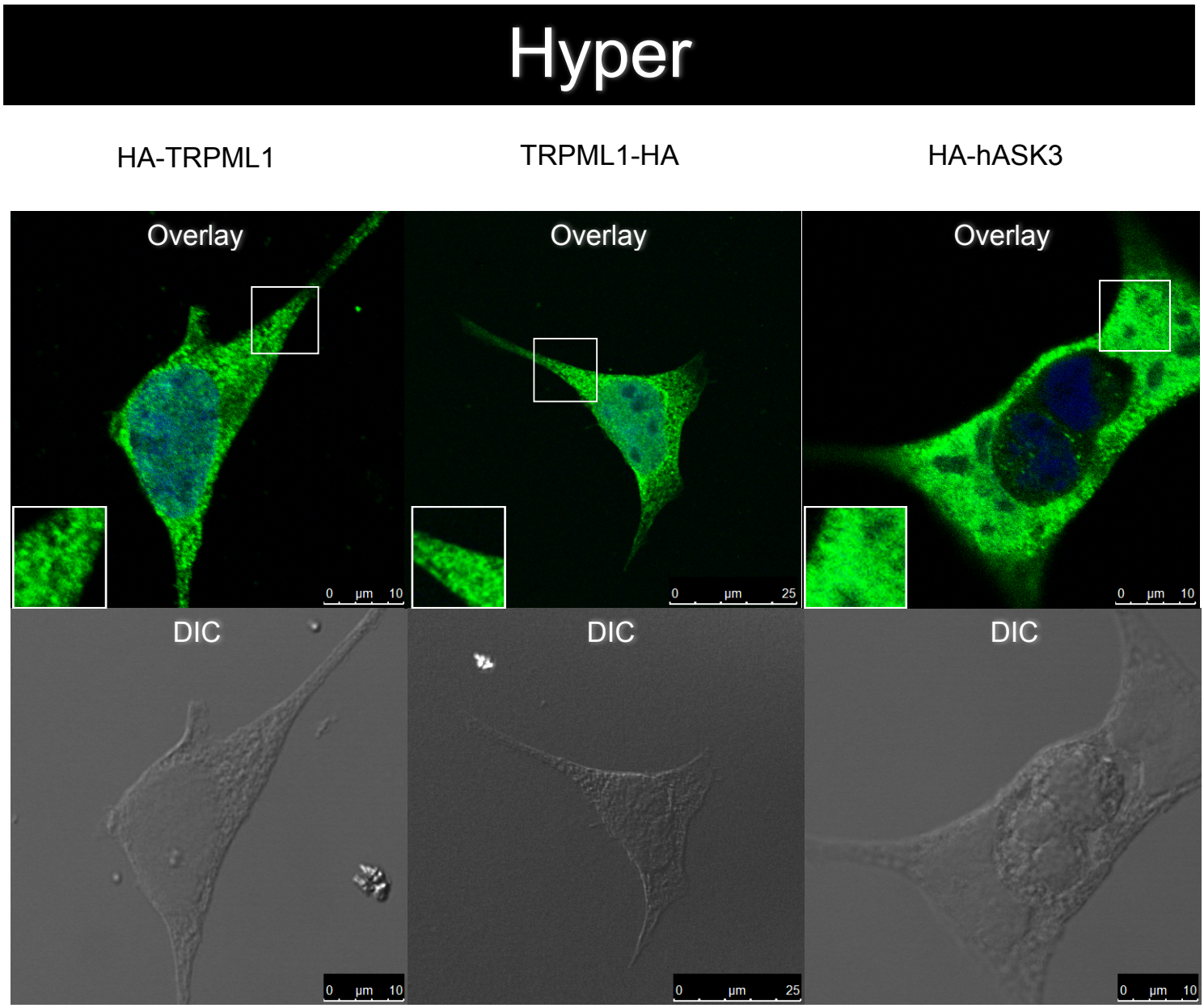
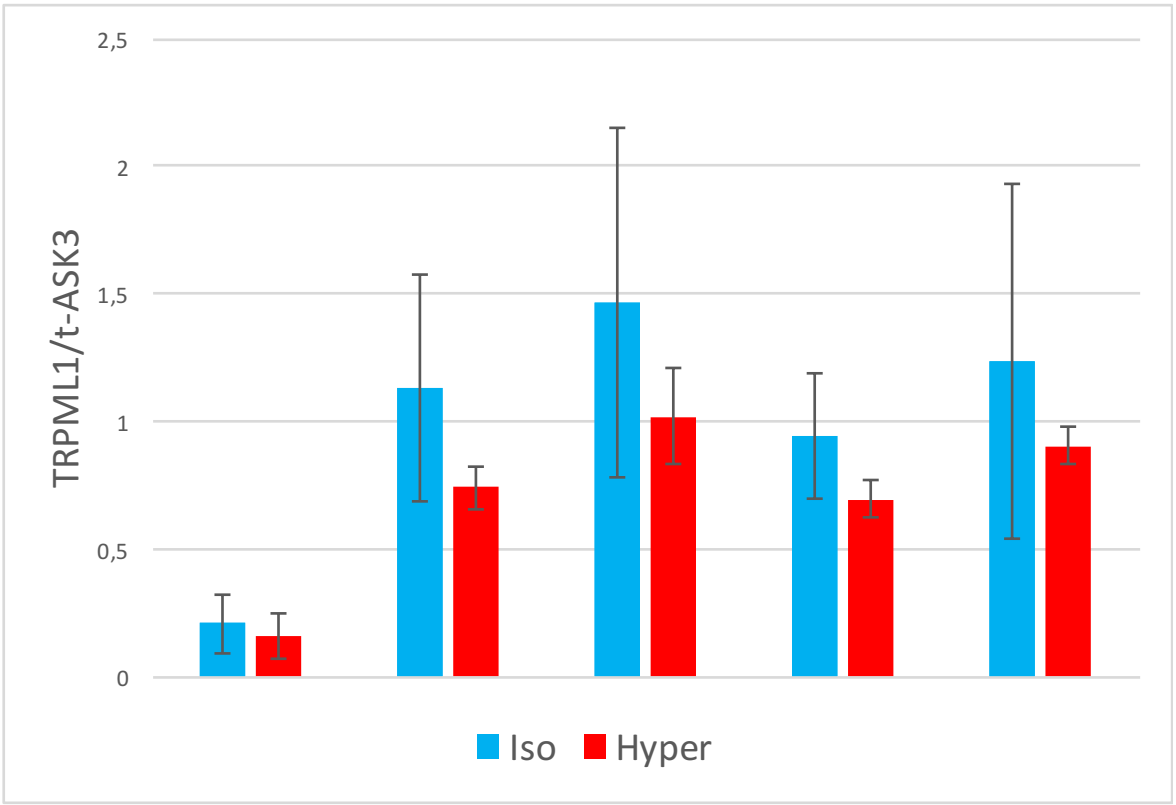


Fig 3. This is HA-TRPML1, TRPML1-HA and HA-hASK3 detected by immunofluorescence after 10 min under hypertonic stress. DIC is included to show the real position of the cell and that it is confluent with the immunofluorescence pictures.

As you can see, TRPML1 is localized mostly in the cytoplasm around the round shape structure (nucleus) under isotonic stress, but it is also seen diffusely in the nucleus under hyper-osmotic stress. ASK3 is localized in both nucleus and cytoplasm under isotonic condition, but solely in the nucleus under hypertonic stress. According to previous reports, TRPML1 will be in the lysosome and endosome, which localizes in the cytoplasm. However, one would have to check co-localization with lysosome and endosome markers. The important point is that over-expression does not appear to produce TRPML1 aggregate. The result for ASK3 was almost as expected, in iso-condition its spread diffusively inside the cell and in hyper-condition it forms round shape structurer. The reason for this behavior is unclear.

6.3 Experiment nr 3: The effect of TRPML1 (over expression) on ASK3 (over expressed) inactivation

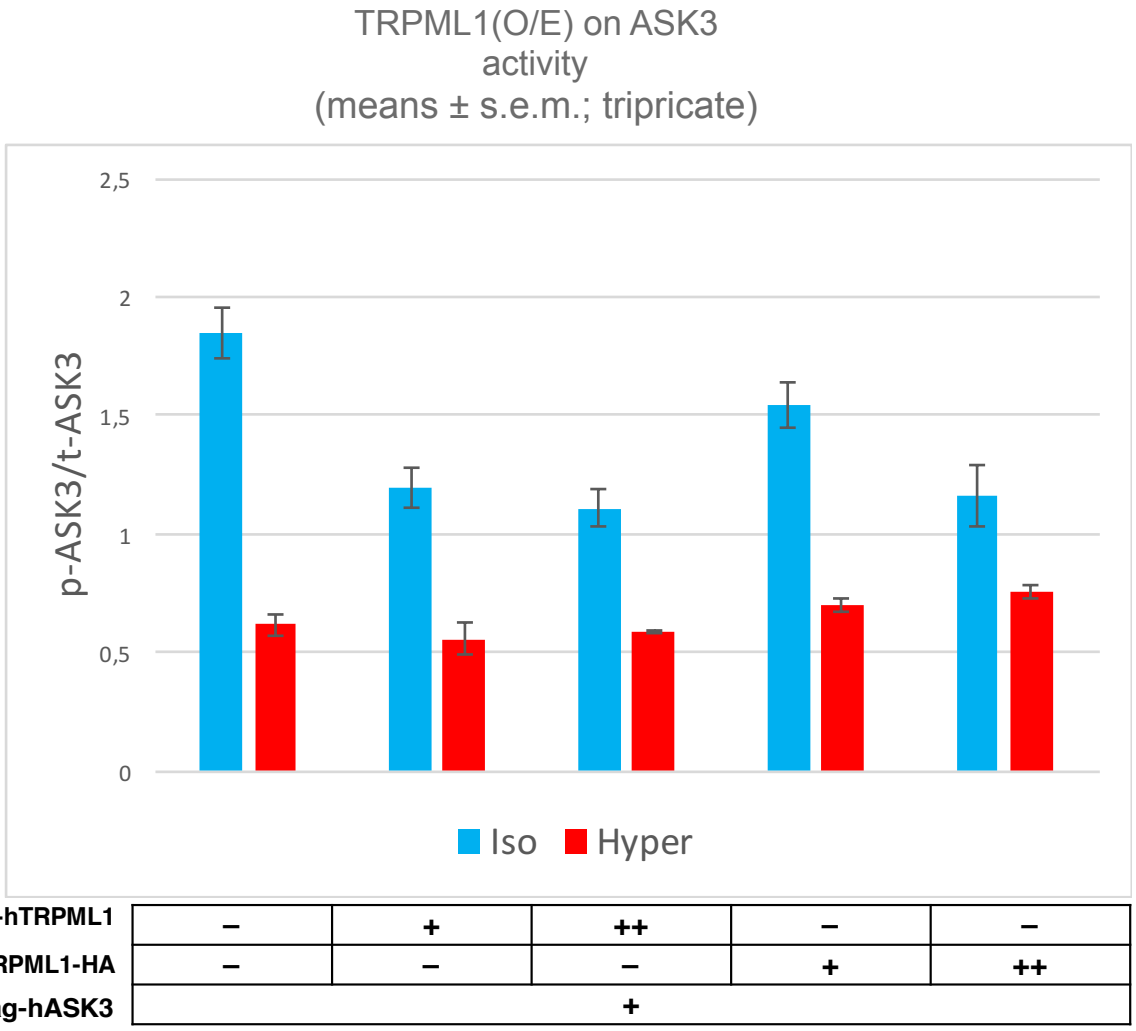
After observing the sub-cellular localization of TRPML1 and that it did not aggregate when over-expressed, we evaluated whether or not TRPML1 affects ASK3 inactivation. The hypothesis is that more TRPML1 leads to stronger inactivation of ASK3 under hyper-osmotic stress. The results are verified by high content analysis.



HA-hTRPML1	–	+	++	–	–
hTRPML1-HA	–	–	–	+	++
Flag-hASK3	+				

Fig 4. This shows the co-transfection level by high-content analysis with the antibodies for FLAG-tagged ASK3 and HA-tagged TRPML1.

We confirmed that the ratio of TRPML1 over ASK3 was low in negative controls. In other samples there is an increase in both TRPML1. HA-TRPML1 and TRPML1-HA follow the same pattern. The over-expression of TRPML1 was successful.



HA-hTRPML1	–	+	++	–	–
hTRPML1-HA	–	–	–	+	++
Flag-hASK3	+				

Fig 5. This is ASK3 activity level detected by high-content analysis with the antibodies for Flag-tag and phosphorylated ASK3.

This graph shows that in iso-condition, pASK3 decreases with higher amount of TRPML1. For hyper-condition the difference is less distinct. If pASK3 decreased with increased TRPML1 under hyper-osmotic stress, the hypothesis would have been confirmed.

6.4 Experiment nr 4: Create sgRNA and established TRPML1-KO cell line

When considering the siRNA knockdown data by Dr. Watanabe, it seemed logical to make a condition where cell has no TRPML1 to evaluate the necessity for TRPML1 in ASK3 inactivation. And so, CRISPR-Cas9 was used to create knockout cells.

We started with designing sgRNA, which is the guide RNA for Cas9. TRPML1 is located in chromosome #19 and PAM sequence was observed in the TRPML1 genome. We selected 4 sequences in the first and second exon for target. These sgRNA will guide Cas9 to the sequence of interest and induce double-strand breaks. The cell will then try to fix the break through non-homologous end joining (NHEJ) which is prone to errors. These errors will result in the knock-out of the gene.

When designing 4 sgRNA, the 1st sgRNA unfortunately turned out to be outside the TRPML1 sequence, but it was used anyway just to observe and to compare the results.

The purpose of the GFP reconstitution assay is to confirm if the sgRNA works. GFP reconstitution assay involves incorporation of the target gene into a EGxxFP vector and then transfecting cells with this vector and sgRNA (cells already containing Cas9). If the sgRNA works it will help Cas9 to induce double strand breaks in the sequence, so that EGFP joins together, and this will result in light being emitted. All cells are also transfected with RFP to confirm if transfection worked, but also to be able to compare EGFP and RFP.

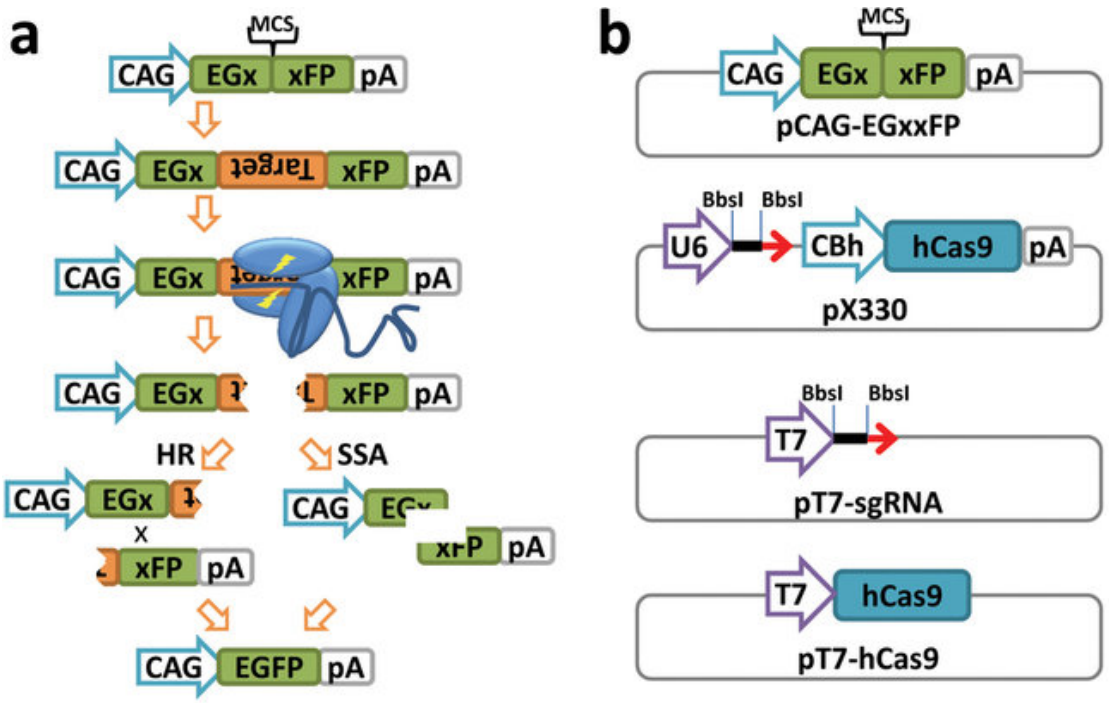


Fig 6, This picture explains the process of Ca9 inducing a dubble strand break in the DNA [11].

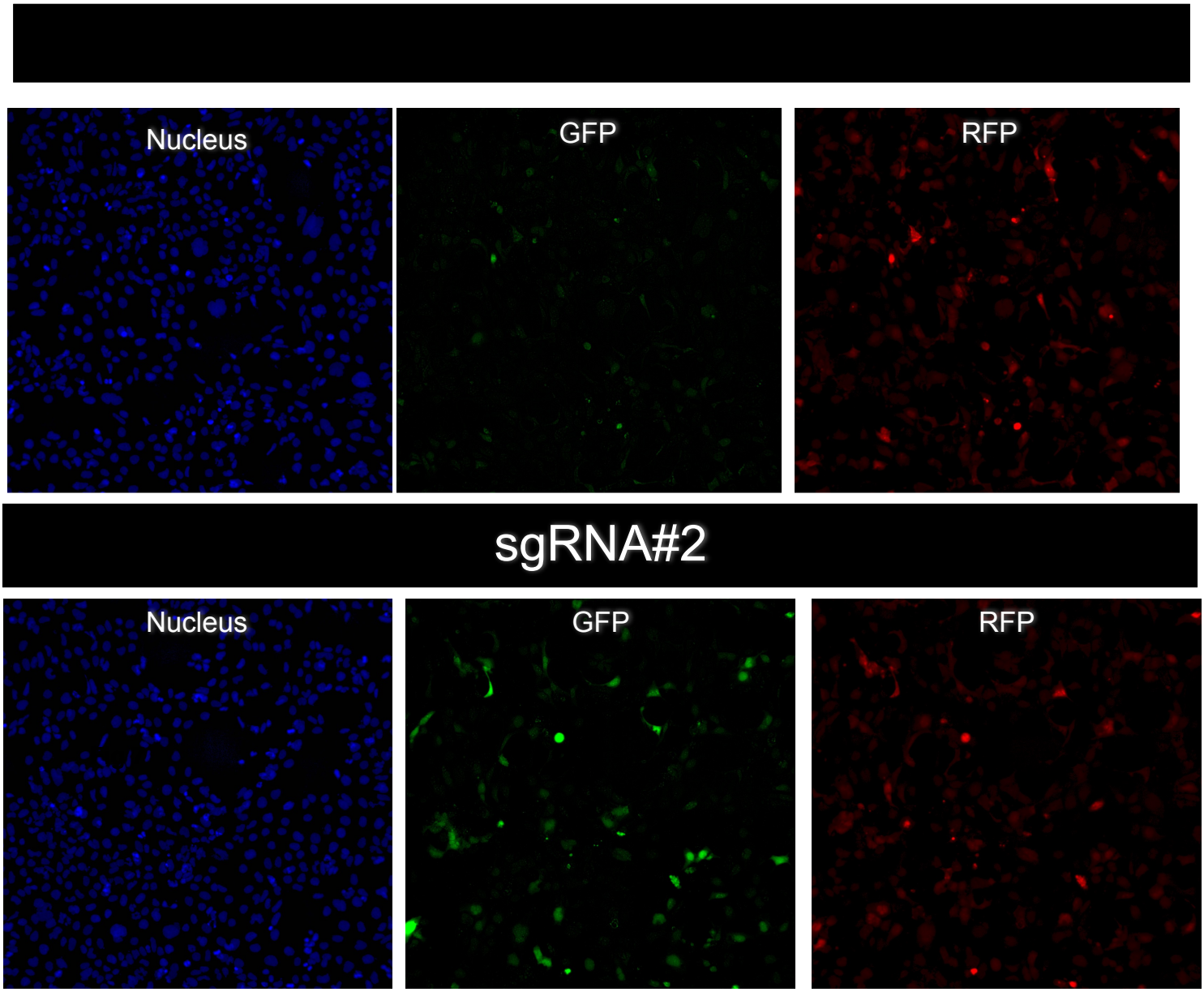


Fig 7, Shows detection by cell-insight for nucleus, EGFP and RFP. In negative control there is now sgRNA.

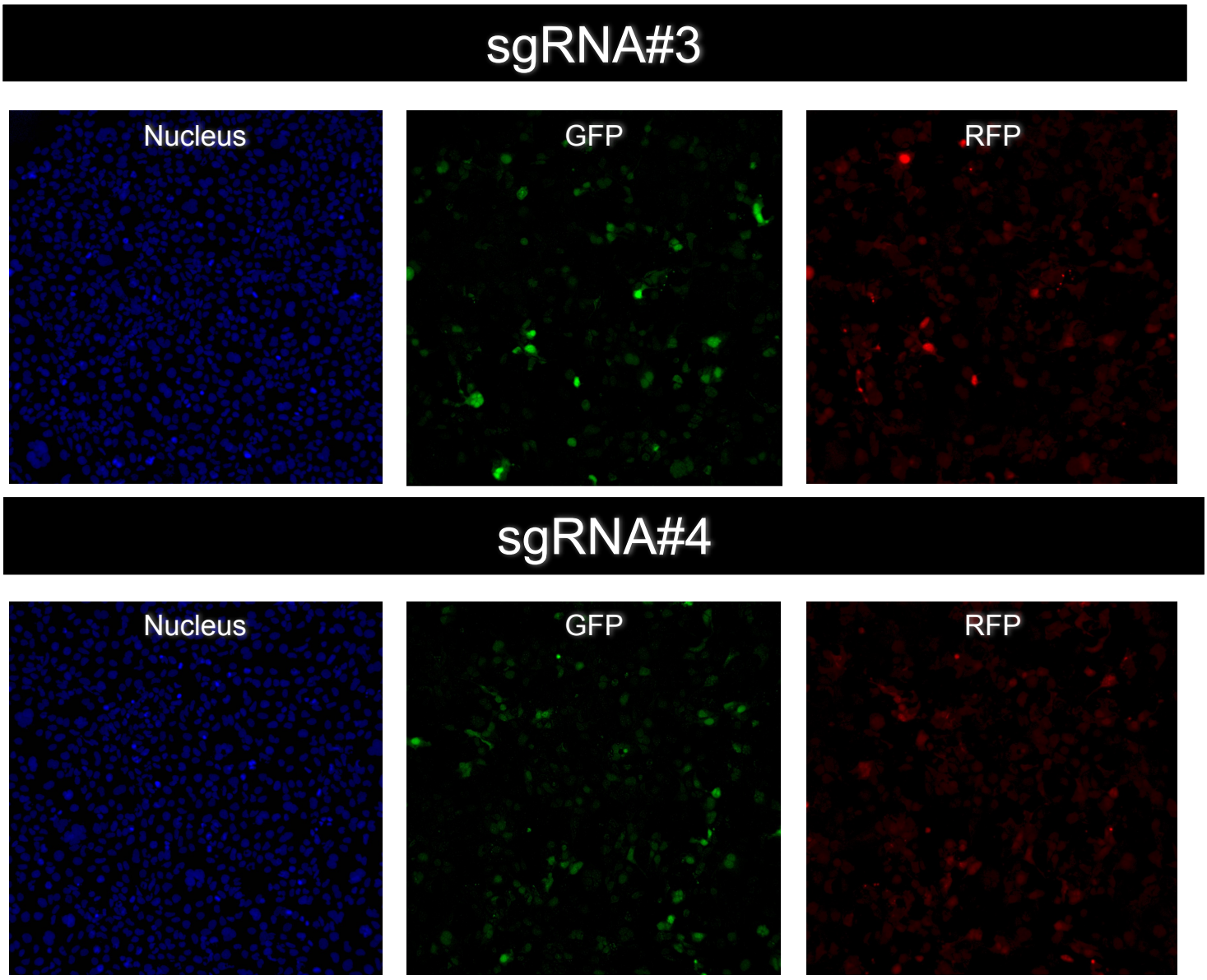


Fig 8, Shows detection by cell-insight for nucleus, EGFP and RFP for sgRNA#3 and sgRNA#4

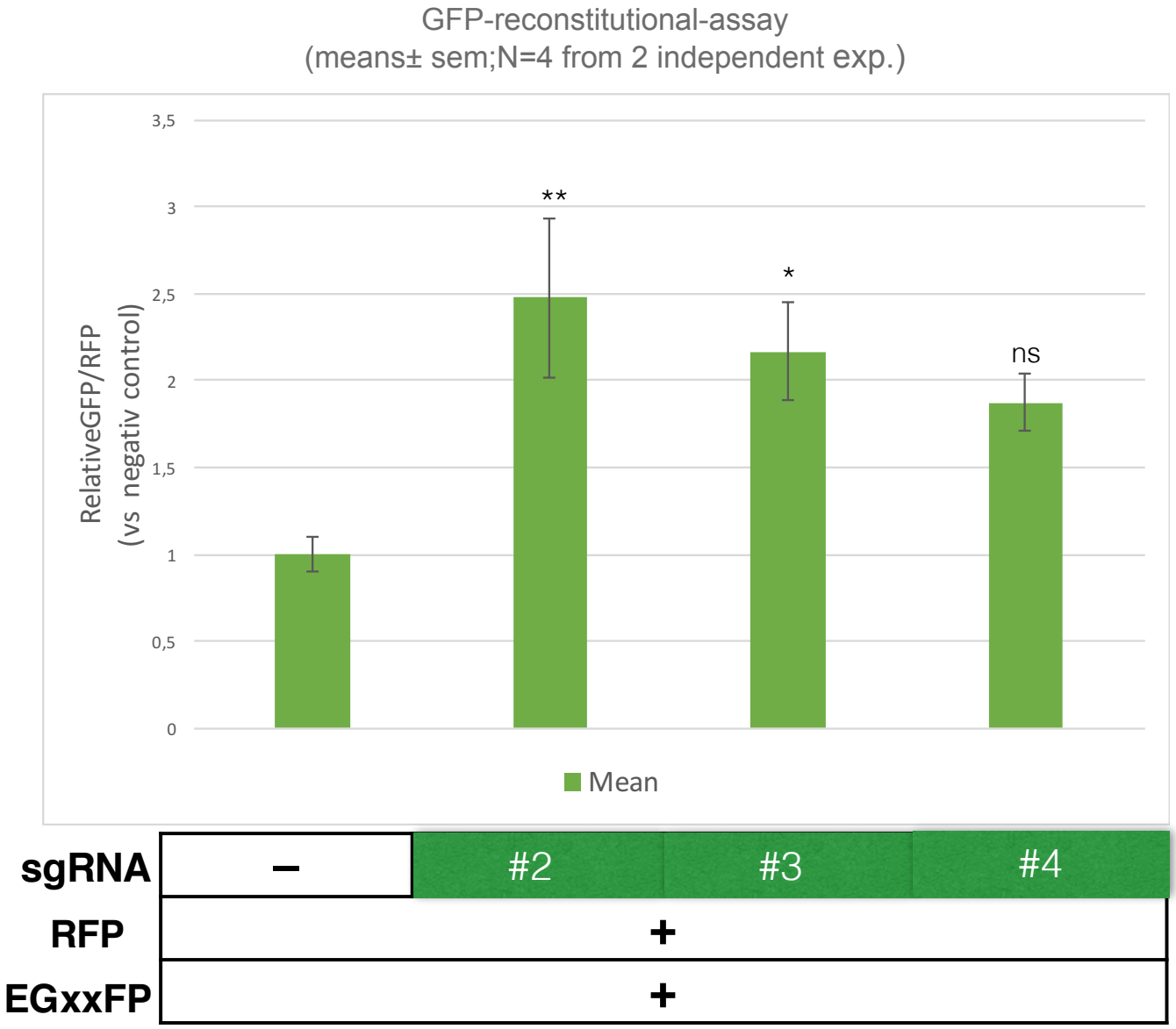


Fig 9, Shows the calculation relativeGFP/RFP for sgRNA#2-#4. Two stars means that the value is significant with P value under 0.01. And one star is P value under 0.05. NS stands for non-significant.

The ratio between GFP and RFP is lower in negative control than in sgRNA2 to sgRNA4. This means that the sgRNA is working and that more GFP is expressed in those wells than RFP. The Relative values were calculated and normalized against the mean value of negative control, in order to make the standard deviation narrower. p-values were calculated by ANOVA and Dunnett's test using RStudio.

The results were as follows: sgRNA2 (below 0.01) and 3 (below 0.05) are significantly different from negative controls, but sgRNA4 (above 0.05) shows only a tendency to be different.

TRPML1 KO cell line establishment:

After finding positive results in experiment nr 4, the next step was to try to establish "tetracycline-induced" TRPML1 KO HEK cells. Tetracycline, if added later will remove the tet-repressor of the Cas9-gene, inducing the cells to start expressing Cas9 and becoming KO-cells.

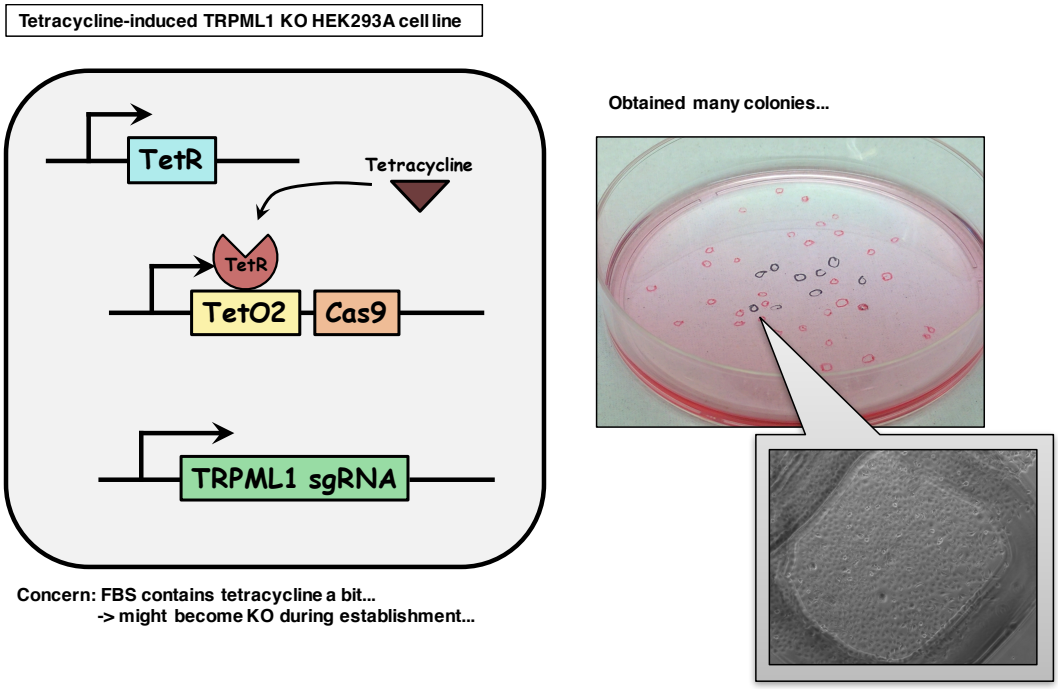


Fig 10, This is an illustration of how tetracycline can remove the tet-repressor from the Cas9 gene in the cells. Making them express Cas9 and be able to KO TRPML1. It also shows the cell colonies obtained.

6.5 Experiment nr5: Transient TRPML1-KO affects pASK3 in hyper-osmotic stress (western-blot)

Because the KO establishment takes a long time, the necessity for TRPML1 for ASK3 inactivation had to be evaluated by partly knockout protocol. This means the experiment is conducted with methods that can not assure the complete KO of TRPML1. The hypothesis is that if TRPML1 is necessary for inactivation of ASK3 under hyper-osmotic stress, ASK3 will still be active under hyper-osmotic stress in TRPML1-knock out cells

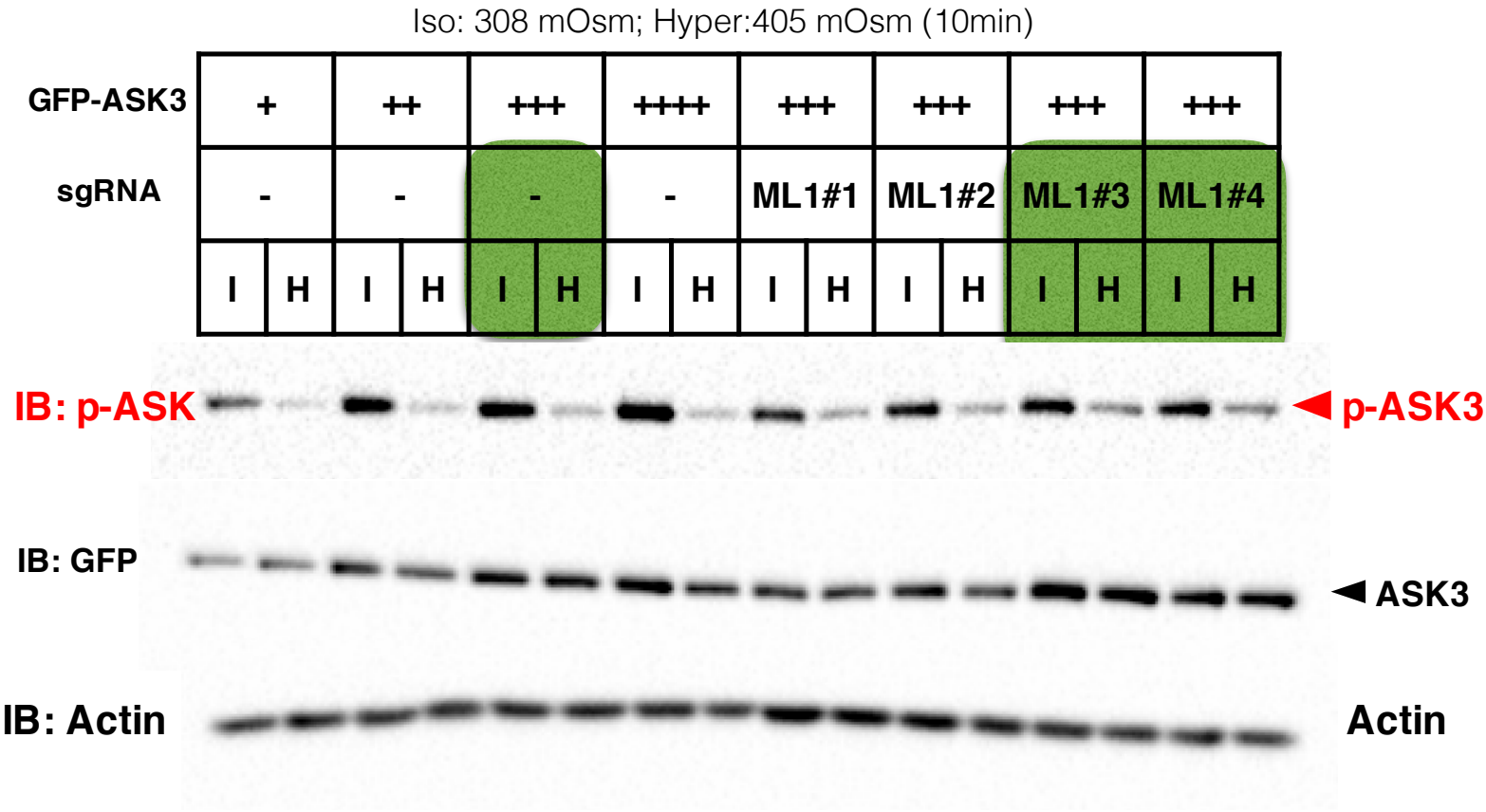
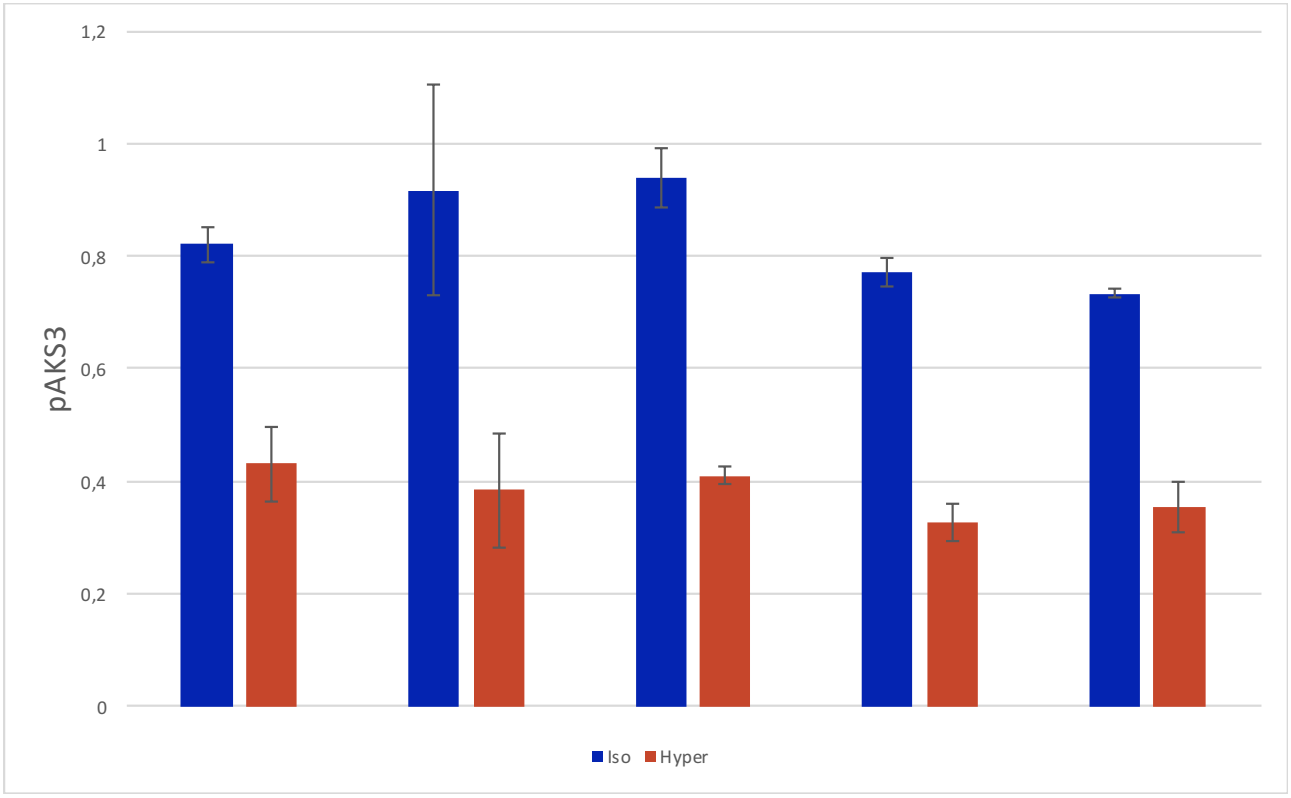


Fig 11, In the first 8 wells, only GFP-ASK3 is transfected. In the last 8 wells sgRNA#1-#4 is cotransfected with a continuous amount of GFP-ASK3. Each well is subjected to Iso och hypertonic stress for 10 min. Actine is also desplayed to show that the cell amount is the same in al the wells and not affecting the results.

In observing the image above, compare the highlighted areas. To be able to compare results for amount of pASK3 in KO and controls, the amount of total ASK3 must be the same. It is then clearly seen that pASK3 activity is increased under hyperosmotic stress when TRPML1 is knocked-out.

This agrees with the hypothesis that if TRPML1 was necessary for inactivation of ASK3 under hyper-osmotic stress, then ASK3 will still be active under hyper-osmotic stress in TRPML1-knock-out cells. This also indicates that TRPML1 might be the upstream regulator of ASK3.



Tomato ASK3	**+**	**+**	**+**	**+**	**+**
TRPML1-sgRNA	**–**	**#1**	**#2**	**#3**	**#4**

Fig 12, Shows the amount of pASK3 under hypertonic and isotonic condition. Also with the presence in well 2-5 of TRPML1-sgRNA to try to KO TRPML1 gene. Tomato-ASK3 was used to gain a different color (red) for Cell-insight to measure. TRPML1.sgRNA (green)

In the next experiment, results was obtained by high-content analysis with almost the same procedure as the previous experiment. Unfortunately, these results does not support the hypothesis. There is no large difference in amount of pASK3 in hyper-osmotic stress between negative controls and the cells where the TRPML1 was knocked-out. It was expected that an increase of pASK3 in hyper-osmotic stress was to be seen when TRPML1 was knocked-out, as in the experiment above.

7. Discussion

To summarize the above results, constructing and over-expressing the TRPML1 vector was successful. The localizations of TRPML1-HA, HA-TRPML1, and HA-hASK3 were observed in cells and it was concluded that they were not aggregated. TRPML1 and ASK3 were over-expressed in order to see how this affected the inactivation of ASK3. In isotonic condition, ASK3 decreases with higher amount of TRPML1, but in hypertonic condition no clear difference could be observed. sgRNA was created for CRISPR-Cas9 and the development of a TRPML1 knock-out cell line was set in motion.

We examined how a transient TRPML1 knock-out would affect the inactivation of ASK3 under hyper-osmotic stress. Results showed that when TRPML1 was knocked-out, ASK3 activation was increased under hypertonic stress.

7.1 Experiment nr 1: Expression vector for TRPML1

In the first experiment (Fig. 1), the expression vector for TRPML1 was created and over-expressed in HEK cells. A monomer of TRPML1 was observed at 65KDa [12]. It was noted that it is a non-optimal way to see how TRPML1 affects pASK3 under hyper-osmotic stress. Expressing ASK3 and TRPML1 on the same membrane will cause them to overlap, affecting the detection results. Therefore, I chose to detect TRPML1 and ASK3 by high-content analysis. One relevant question is why the upper band appears, using normal SDS-PAGE? The oligomer should not be present. The answer to this question is that TRPML1 transmembrane domain might make it harder to denature [13]. One could attempt to add more Ditiotreitol (DTT) or lower boiling temperature. If the oligomer then disappears, it would be good to co-transfect with ASK3 since they would not overlap. The oligomer would have disappeared and the part of the membrane involved would show ASK3 without interference.

7.2 Experiment nr 2: TRPML1 localization

TRPML1 was localized to round-shape structure (Fig. 2 and 3). According to previous reports, the early endosome and lysosome should be the granular structure outside of the nuclear membrane [12]. A note to this experiment would be that if this experiment is repeated, one could try to expose the cells to osmotic stress for a shorter time period. In this experiment, the cells was in the hyper- or iso-tonic condition for 10 min. This may be to long for a change in localization of TRPML1 to be detectable. It would have been interesting to see if TRPML1 under hyper-osmotic stress would localize to the cell membrane. If so, it is more possibie that TRPML1 is the hyper-osmotic sensor HICC. One theory regarding the HICC is that it has to be localized to the cell membrane at some point, otherwise it would be hard for it to detect changes in osmolarity outside the cell.

7.3 Experiment nr 3: The effect of TRPML1 (over expression) on ASK3 (over expressed) inactivation (Fig 4 and 5)

The reason why any change could not be observed in hyper-condition might be due to that cell insight cannot measure lower amounts of data. This is to say that the hyper osmolarity was not strong enough to invoke a change in pASK3. To prove this, one would have to do the same experiment again with higher osmolarity to see if one can obtain a sharper decrease in pASK3 in hyper-osmotic stress.

Other possibilities might be that pASK3 only decreases in isotonic stress when TRPML1 is present. The reason might be that endogenous TRPML1 activation is sufficient under hyper-osmotic stress. To prove this one could treat the cells with TRPML1 agonist and observe similar results.

7.4 Experiment nr 4: Create sgRNA and established TRPML1-KO cell line (Fig 6, 7, 8 and 9)

GFP was brighter for the sgRNA [14] than in controls which indicates that the experiment was successful.

Establishment of TRPML1 KO cell line: (Fig. 10)

This experiment is unfinished and will continue. If successful, one could use the same kinds of cells in one experiment with inducing KO in one dish of cells and leaving one dish with control normal cells. This would have advantages since one would not need two different cell lines for one experiment, leading to a more equal condition and easier comparison of results. One concern for this experiment is that the cell medium contains FBS, and this in turn contains some tetracycline. This might affect the cells and bring about immediate knock-out. If this happens it would still be possible to use the cells, which would be very useful for future experiments on TRPML1 effect on ASK3.

7.5 Experiment nr 5: Transient TRPML1-KO affects pASK3 in hyper-osmotic stress (western-blot)

For the western-blot data in Fig. 11, total ASK3 does not change much, but when comparing with pASK3 it is clear that TRPML1-KO pASK3 activity increases. Looking at the high content analysis data in Fig. 12, there is no large difference in pASK3 in hyper-osmolarity between negative control and the sgRNA (KO-TRPML1). The reason might be a non-total KO of TRPML1. We only waited 72h after transfection, which may be insufficient to get rid of already produced TRPML1. Since it is a membrane protein with slow turnover rate, also mRNA degradation is needed and how long this takes is unknown for TRPML1. To confirm the result, the experiment needs to be repeated, but this time wait longer or use KO-TRPML1 cells. The results can also be due to insufficient number of the cells. The cells analyzed was only around 100, but normally 1000 is required. Fewer cells make it

hard to see a difference. Also transfection rate may be low, since after waiting for 72h ASK3 might already be degraded.

To avoid the above complication, one could do the transfection twice, first with sgRNA and after a few days with ASK3. But if doing so one would not gain the benefits of dual transfectio. This also makes it more likely to conclude that all cells contain both vectors.

The above results are from 3 months of lab work and are not enough to draw any definite conclusions about the regulation of ASK3. Even so, I think my work is promising and worth to continue. The experiments need to be repeated and confirmed. If my results are valid there is a good chance that TRPML1 is the regulator of ASK3 under hyper-osmotic stress. The field is new and the reference background is still limited.

8. Conclusions

The elucidation of ASK3 regulation under hyper-osmotic stress might help to develop new strategies to treat hypertension. Hypertension affects a large portion of the population suffering from cardiovascular diseases. Treatments based on the principles could offer an useful alternative. The project is still early but shows promise, eve though the experiments described need to be reproduced. My results suggest TRPML1 to be the upstream regulator of ASK3.

9. Populärvetenskaplig sammanfattning

Förändringar av saltbalansen (s k osmolaritet) i kroppen kan innebära en fara för vissa celler. Cellerna har därför utvecklat system för att känna av och reagera på dessa förändringar för att bibehålla jämvikt och storlek. Om saltkoncentrationen utanför cellen ökar (hyperosmotisk stress), svarar cellen med att försöka utjämna skillnaden och pumpa ut vatten vilket leder till att cellen minskar i storlek. Det motsatta händer om miljön utanför cellen har för låg salthalt (hypoosmotisk stress). När miljön kring cellen sedan är återställd behöver den återgå till sin normala storlek för att inte bli skadad eller dö.

Apoptosis signal regulation kinas 3 (ASK3) tillhör familjen MAP3K och uttrycks framför allt i njurarna. Det har visats att ASK3 är viktig i cellerna för återställandet av deras volym efter hyper- eller hypoosmotisk stress. Man har även sett i studier att möss utan ASK3 fick högt blodtryck vilket innebär att ASK3 kan påverka blodtrycket.

Syftet med detta projektet var att studera vad som reglerar ASK3 under hyperosmotisk stress. ASK3 ser ut att spela stor roll för regleringen av blodtrycket. Forskning kring hur ASK3 regleras kan leda till nya strategier för hur man kan behandla högt blodtryck. I tidigare experiment utförda på samma labb som jag befann mig på, har man funnit tecken på att transient receptor potential cation channel (TRPML1) kan vara den jonkanal som reglerar ASK3 under hyperosmotisk stress. Jonkanaler sitter i bland annat i cellmembranet och tillåter att vissa salter kan transporteras in och ut i cellen.

Det är viktigt att man fortsätter forska inom detta område. Tydliggörande av regleringen av ASK3 kan leda till utvecklande av nya metoder för att behandla högt blodtryck. Högt blodtryck är en vanlig sjukdom som i sin tur är en riskfaktor att utveckla hjärt- och kärlsjukdomar, såsom hjärtinfarkt.

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