27 June 2014

The role of the Hippo-YAP pathway in tumorigenesis

Author: Eldert van der Ploeg (S2147602)

Faculty of mathematics and natural sciences
Biomedical sciences, Life Science and Technology,
University of Groningen, Netherlands

Supervised by: Prof. dr. F.A.E. Kruyt
Department of medical oncology
Faculty of medical sciences

Abstract

In this essay an overview is shown of the function of Yes Associated Protein (YAP) in the Hippo pathway and its role in tumorigenesis. The Hippo pathway has been described as an important regulator of cell growth. The pathway takes its name form one of its key signaling components, the protein kinase Hippo (Hpo). Mutations in this gene leads to tissue overgrowth or a hippopotamus-like phenotype in the D. Melanogaster. The Hippo pathway is highly conserved throughout the evolution. The hippo pathway and its effector, YAP, have emerged as important regulators of organ size and proliferation. Amplification of YAP has been found in multiple different tumors types and causes multiple oncogenic properties in cell lines. YAP overexpression stimulates proliferation and increases anti-apoptotic signals, increased levels of YAP can also can induce epithelial to mesenchymal transition (EMT). Paradoxically YAP is not only described as potent oncogene but also as tumor suppressor gene, in some tumors YAP is down regulated. Down regulated YAP results in suppressed anoikis, increased migration and invasiveness. When YAP is amplified it also has an important clinical relevance since high levels of YAP are associated with poor overall survival in patients with ovarian cancer and Hepatocellular carcinomas. YAP can also serve as potential direct or indirect target for anti-tumoral effect. When YAP is amplified microRNA-375 can directly target YAP and causes a decrease in proliferation and migration. γ-secretase inhibitors causes also decreased proliferation and migration by targeting Notch which is activated by YAP. Also when YAP is down regulated inhibition of Macrophage Stimulating 1 (MST1) leads to restoration of YAP levels and causes more apoptosis and a decreased organ size. This overview stresses the importance of the Hippo-YAP pathway in tumorigenesis.

Key words: Hippo pathway, YAP, cancer, TEAD, oncogene, tumor suppressor, proliferation, apoptosis.

Table of contents

- Abstract Page 1

- Introduction Page 3

- The Hippo pathway and the function of YAP Page 4

- The role of YAP in tumorigenesis Page 6

- YAP as a prognostic marker Page 7

- YAP as a potential (in)direct target Page 8

- Discussion Page 10

- References Page 13

Introduction

During the development of an animal exponential proliferation of cells take place. When animals enter adulthood this exponential proliferation has to be controlled and cell divisions has to be limited to only maintenance of adult tissues. When this control of proliferation is impaired an unrestricted cell division can be observed. Unrestricted cell division is one of the hallmarks of cancer and often involves genes that normally regulate proliferation and apoptosis. Efforts to identify regulators of proliferation that serve as rate-limiting factors for cancer progression are therefore very important (1).

Genetic screens used in D. Melanogaster have identified many genes that have mammalian homologous which functions as oncogenes or tumor suppressors (2). The rapid pace of performing genetics in D. Melanogaster have facilitated unraveling of the organization of interactions between growth regulators into pathways and networks of control. An example of a pathway discovered by genetic research in D. Melanogaster is the Hippo signal transduction pathway. The pathway takes its name from one of its key signaling components, the protein kinase Hippo (Hpo). Mutations in this gene lead to tissue overgrowth, or a hippopotamus-like phenotype in the D. Melanogaster (1).

The human homologue of the hippo pathway and its effector, the transcriptional co-activator Yes-associated protein (YAP) have emerged as major regulators of organ size and proliferation (3). Although the membrane components of the Hippo pathway have not been completely discovered it is clear that the pathway is regulated by cell-cell density (4). As mentioned before the Hippo pathway was first described in the D. Melanogaster and is highly conserved throughout the evolution (5). Similar to many pathways involved in growth control, Hippo signaling is important in development and is a vulnerable target for misregulation in cancer. Mutations of all of the components in the pathway have been found in human cancers, or have been found to result in tumorigenesis in mice (1). Amplification of YAP has been identified in mammary tumors and its overexpression causes multiple oncogenic properties in cell lines (6)(7). Amplification of YAP is shown is several human cancers, such as breast, ovarian, liver and colorectal cancers indicating the possible oncogenic function of YAP (8). YAP overexpression stimulates proliferation and increases anti-apoptotic signals in humans. Interestingly Yap overexpression in mice causes a dramatic increase of liver size and can eventually lead to tumor growth (9). These observations have established the importance of YAP in the Hippo pathway in the development of human cancer.

Accumulating evidence supports the role of YAP as organ size regulator and as oncogene. So it is important to know the exact mechanism of YAP in the Hippo pathway to understand the role of YAP in tumorigenesis. Several questions are addressed in this review to give an overview of the function of YAP in the Hippo pathway and its role in tumorigenesis. What are the most important components and functions of the Hippo pathway and what is the function of YAP in this pathway? What is the role of YAP in tumorigenesis? Can it be used as prognostic marker and what are potential therapies to target Yap for anti-tumoral effect? To answer these questions literature research is used.

The Hippo pathway and the function of YAP

Most of the information of the Hippo pathway has been discovered in the D. Melanogaster. Nearly all of the components of the pathway were discovered in genetic screens; when these genes were inactivated the cells showed an excessive tissue growth of epithelial cells and inhibition of apoptosis (1). This suggests that the Hippo pathway plays an important role in the control of cell growth. For a schematic presentation of the Hippo pathway see figure 1. The first two components that were discovered in the D. Melanogaster were the kinases Warts (Wts) and Hippo (Hpo). Hpo can phosphorylate and activate Wts. Two proteins, Salvador (Sav) and Mob as tumor suppressor (Mats), facilitate the phosphorylation of Wts. Once activated Wts phosphorylates and inhibits Yorkie (Yki). Yki normally regulates the transcription of target genes that are involved in cell proliferation and apoptosis by binding with transcription factor scalloped (Sd) (1). Upstream of Hpo two membrane associated proteins, Merlin (Mer) and Expanded (Ex) have been described in the pathway. Both proteins contain a domain that interacts with several cytoskeletal and transmembrane proteins such as the protocadherin Fat. This suggests that Mer and Ex may transmit signals from the membrane (10)(11). Hamaratoglu et al have shown that mutations in Mer and Ex leads to tissue overgrowth and they discovered that Mer and Ex stimulates the phosphorylation and activation of Wts by Hpo (12). Willecke et al discovered that Fat mutant cells continued to proliferate after wild-type cells stopped proliferating and that fat mutant cells deregulated hippo target genes (13). They elucidated the transmembrane protein Fat as the link between the Hippo pathway and an extracellular ligand or signal. So when the cells have contact with an extracellular ligand or signal Fat is activated, which on his turn activates Ex and Mer, next Hpo phosphorylates and activates Wts and Yki is phosphorylated and inhibited by activated Wts. Inhibited Yki does not longer promote cell proliferation and anti-apoptopic signals and tissue growth is controlled in the D. Melanogaster.

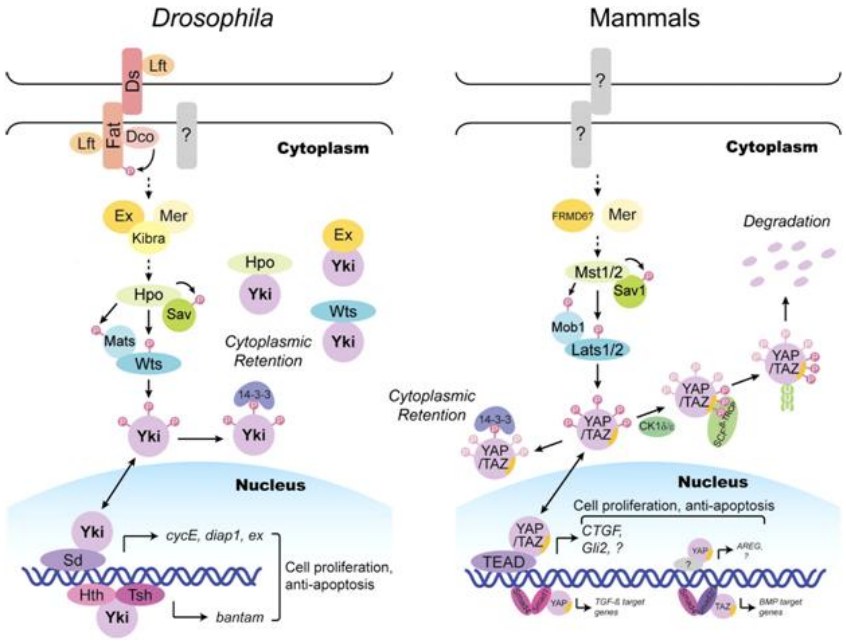

Figure 1: The hippo signaling pathway

On the left is the Hippo pathway in the D.Melanogaster shown, and on the right the Hippo pathway in mammals. The Hippo pathway is highly conserved throughout the evolution. Phosphorylation of the Hippo pathway component Yki/YAP leads to cytoplasmic retention/degradation of the protein. Non-phosphorylated Yki/YAP leads to cell proliferation/anti-apoptopic signals. Adapted by Zhao et al. (2010) (14).

As mentioned earlier, components of the Hippo pathway are highly conserved throughout the evolution, most of these components have direct homologues with mammals (see also figure 1). Macrophage stimulating 1 (MST1/2, homologue of Hpo) phosphorylates Large tumor suppressor kinase 1/2 (LATS 1/2, homologue of Wts) which in his turn phosphorylates and inhibits YAP/TAZ (homologue of Yki). Inhibited YAP does not longer promote cell proliferation and anti-apoptosis signals in mammals. The most striking evidence of the conservation

throughout evolution is that the mammalian homologues of the Hippo pathway can functionally rescue the corresponding D. Melanogaster mutants in vivo, This suggests a functional conservation of these genes (5).

When activated the Hippo pathway inhibits YAP, LATS 1/2 inhibits YAP by Serine 127 (S127) or Serine 381 (S381) phosphorylation and cytoplasmic retention occurs (14). Therefore YAP cannot longer bind its nuclear target transcription factors. When Lats1/2 phosphorylates S127 only cytoplasmic retention takes place, and when LATS1/2 phosphorylates S381, YAP is more prone to be phosphorylated by CK1δ/ε and is subsequently ubiquitinated and degraded. Zhao et al. present the explanation that phosphorylation of S127 could serve as a short term inhibition of YAP and phosphorylation of S381 could serve as long term irreversible inhibition (14). Dysregulation of both mechanisms could lead to oncogenic transformation. The S381- initiated degradation of YAP is not conserved in the D. Melanogaster homologue Yki.

To investigate how YAP regulates cell proliferation and anti-apoptotic signals Zhao et al performed transcription factor screens. They found that YAP could interact with the transcription factor TEA- domain (TEAD) (8)(14). YAP can serve as a co-activator of the TEAD transcription factor. YAP does not have DNA binding activity and TEAD plays a major role in mediating the binding of YAP to gene promoters. They found that together the YAP-TEAD complex promotes proliferative and survival programs. In the D. Melanogaster the homologue complex promotes cell proliferation by activating cyclin E and Inhibitors of Apoptosis Proteins (IAP). These genes were not significantly induced by YAP in mammals, which indicates that there might be different genes in mammalian cells to mediate YAP function. Zhao et al found that the gene Connective Tissue Growth Factor (*CTGF*) is an important downstream target of YAP-TEAD complex, which is a matricellular protein and has an important role in different biological processes such as cell adhesion, migration and proliferation (8). Besides *CTGF* there are other genes to regulate cell and tissue growth, which still has to be elucidated.

During normal adult homeostatic conditions where there is cell-cell contact, YAP is mostly phosphorylated by LATS1/2. Therefore, YAP does not longer enter the cell nucleus and cannot stimulate cell proliferation and anti-apoptotic signals. Interestingly in adult stem cells, which normally reside in well-defined, organ-specific progenitor cell compartments, there are several observations that link the hippo pathway activity to stem cell function. For example YAP and TEAD expression are increased in compartments containing stem/progenitor cells (15). This activation of YAP to progenitor or stem cells compartments indicates that the transcriptional activity of YAP could be important in the maintenance of stem cells in normal tissues. There are several studies that have described YAP to be associated with the regulation of stem cell activity (16).

Micro-RNAs (miRNAs) are important regulators in gene expression at post-transcriptional level. They have been linked to many oncogenes or tumor suppressors. They can play important roles in tumorigenesis by regulating expression of genes that are associated with oncogenic signaling. Liu et al. found that miRNA-375 is involved in the regulation of the oncogene YAP (17). They found that in Hepatocellular carcinomas (HCC) where YAP was overexpressed miRNA-375 was down regulated, suggesting that YAP a target is of miRNA-375. They further examined the effect of miRNA-375 on YAP and they found that miRNA-375 caused a decrease in YAP protein level in two different cell lines. The expression of miRNA-375 also resulted in a decrease of CTGF mRNA levels (17).

Paradoxically Strano et al. have shown that YAP does not only induce anti-apoptotic signals but

can also induce apoptosis in case of DNA damage (18). They showed that YAP could also bind to a region of p73 under physiological conditions. p73 is a nuclear protein which binds to canonical p53 DNA binding sites and can activate transcription from p53 promoters. Activation of p73 can induce apoptosis, growth arrest and differentiation suggesting that YAP can also induce apoptosis instead of only inducing anti-apoptotic signals (18).

The role of YAP in tumorigenesis

As stated before the Hippo pathway plays an important role in organ development and mutations in components of the Hippo pathway have been linked to the development of cancer. The YAP-TEAD transcription factor complex represents a common target of oncogenic transformation. Amplification of the YAP gene locus has been reported at many different tumor types such as medulla-blastomas, oral squamous-cell carcinomas and carcinomas of the lung, pancreas, esophagus liver and mammary gland (19). Also most common solid cancer types revealed widespread and frequent YAP overexpression in lung, ovarian, pancreatic colorectal hepatocellular and prostate carcinomas (19). Interestingly in nearly all cases amplification of YAP also leads to enhanced cellular inhibitor of apoptosis 2 (cIAP2) expression, which is involved in the inhibition of apoptosis suggesting a potential cooperation between YAP and cIAP2 in tumorigenesis (20). cIAP2 and YAP can both act independently as oncogenes however the precise role of the combination of YAP and cIAP2 in tumorigenesis has to be further investigated. The question remains what the direct consequence is of the amplification of YAP in several tumor types.

Camargo et al. investigated the role of Yap in causing increases of organ size (9). To investigate this they generated a transgenic mice in which Yap1 was overexpressed. Activation of Yap of 35 days resulted only in a more than four-fold increase of liver size and increased proliferation in the intestine. This increase in liver size was shown to be reversible and was due to an increase of cell number rather than cell size. In the intestine proliferation was greatly increased when Yap was activated, interestingly proliferation upon Yap activation was not only shown in the crypt regions but was also detected at the villus region and at the tip of the villi. The results of Camargo et al. indicated that the activation of Yap leads to a loss of differentiated cell types in the small intestine. Also they suggested that activation of YAP results in expansion of multi-potent undifferentiated intestinal progenitor cells that retain the ability to resume differentiation upon interruption of Yap expression. Wnt and Notch signaling are both important for the maintenance of undifferentiated progenitor/stem cells in the small intestine. Both are altered by the activation of Yap which could mean that those signaling pathways are responsible for expansion of the stem cells through activation of Yap. Camergo et al. concluded that it is possible that the Hippo pathway deregulation also contributes to tumor development by stimulating the accumulation of undifferentiated progenitor/stem cells in vivo (9).

Overholter et al. showed that in MCF10A human mammary epithelial cells where YAP was overexpressed loss of cell-cell contacts is shown and cells were spread, not clustered and also their morphology had changed (6). This suggests that these cells had undergone Epithelial to Mesenchymal transition (EMT), EMT is a phenotypic switch by which epithelial cells lose cell-cell adhesion and apico-basal polarity and instead acquire motility, invasiveness and resistance to apoptosis (21). EMT is considered important for tumorigenic progression, and Overholter et al. also evaluated the EMT by

examining the expression patterns of epithelial and mesenchymal markers. They showed that mesenchymal markers were up regulated and epithelial markers down regulated. Collectivity all the observations suggests that YAP is able to induce EMT, which is a characteristic of cancer progression. Also they found that YAP overexpression induces more proliferation and inhibition apoptosis in two epithelial cell lines. So the up-regulation of YAP can have a potent oncogenic effect in cells. Also Ota et al. found that TEAD is involved in the growth-promoting and EMT-inducing activities of YAP. They showed that TEAD was involved in 2 other activities of YAP, suppression of apoptosis and cell transformation/tumor formation. This means that as well YAP as TEAD could serve as a possible therapy target for anti-tumoral effect where Yap is up regulated (22).

Interestingly YAP has not only been described as a potent oncogene but YAP has also been implicated as a potential tumor suppressor by binding to p73 and induce apoptosis as shown by Strano et al. (18). Another study investigated if YAP can be a tumor suppressor in breast cancer since a specific deletion of YAP was found in some breast cancers. They did a knockdown of YAP in a breast cell line and they found that knockdown of YAP suppressed anoikis, increased migration and invasiveness (23). This implicates that YAP can functions as oncogene and as tumor suppressor gene in different, but also in the same, tumor types.

YAP as prognostic marker

An important step in the development of more targeted and personalized treatment is identification of molecules involved in the development of cancer and its progression. Since YAP is amplified in a number of cancer types YAP can possibly serve as a prognostic marker. Genetic screens revealed YAP as an oncogene or tumor suppressor candidate however the clinical value of YAP has not yet been explored. The clinical value is important in several cancers YAP is up regulated or paradoxically in some cancers even down regulated.

Hall et al. first found that YAP was not significantly up regulated or less phosphorylated at S127, and that YAP is not grossly misregulated in ovarian cancer (24). However they reasoned that misregulation in inhibition of YAP could be an important and they sought out to find if the YAP cellular distribution was correlated with patient survival in ovarian cancer. They found that YAP in ovarian cancers was predominantly located in the nucleus and not in the cytoplasm. Higher levels of YAP in the nucleus was associated with poor patient survival and low levels of YAP in the cytoplasm was also associated with poor patient survival. The combination of High YAP in the nucleus and low YAP in the cytoplasm gave a significantly lower overall patient survival than the both alone. They concluded that the combination of high levels of YAP in the nucleus and low levels of YAP in the cytoplasm could serve as a strong and independent predictor of disease-specific survival for ovarian cancer. To determine if YAP is an activate driver of ovarian cancer progression they overexpressed YAP, they expected that Hippo pathway cannot longer phosphorylate all the excess of YAP and more YAP would be found in the nucleus. They found that overexpression of YAP lead to more proliferation and decreased apoptosis. Interestingly they also found that overexpression of YAP leads to a decrease in cisplatin-induced apoptosis. To investigate what happened if YAP was down regulated they knocked down YAP, this resulted in that cells were more sensitive to cisplatin-induced apoptosis than control cells. This study has shown that an increase of YAP leads to a cancer-associate phenotype, such as more proliferation and

decreased apoptosis, and reduction of YAP resulted in an increase of sensitivity of chemotherapy in cancer cells. YAP distribution in the cell seems to be a strong predictor of survival from ovarian cancer (24)(25).

Another study examined if YAP can also serve as a prognostic marker in HCC (25). They found that also here YAP was predominantly expressed in the nucleus and to a lesser extent in the cytoplasm. In contrast no YAP was found in the nucleus of the corresponding non-tumor tissue. They also found that YAP expression in HCC tumors was associated with poor cellular differentiation, this supports the concept that YAP can contribute to tumor development by stimulating the undifferentiated cells as described by Camargo et al. (9). In this study YAP was found to be an independent prognostic marker for the decreased overall survival of HCC patients. They also examined if YAP expression was associated with tumor recurrence and disease-free survival. They found that high YAP expression in HCC could was indicative of a potential early disease recurrence, also YAP was an independent predictor of short disease-free survival time for HCC patients (25).

Together these results suggests that YAP has a clinical relevance to be used as an independent prognostic factor for the overall survival in both ovarian cancer as HCC. Also YAP is an independent predictor of short disease-free survival. YAP can serve as a new cancer biomarker, and also therapy can be personalized if YAP expression is increased in the tumor because high expression is associated with a decrease of sensitivity of cisplatin-induced apoptosis in ovarian cancer.

YAP as a potential (in)direct therapeutic target

High levels of YAP correlates with lower overall survival, if YAP is up regulated this leads to more proliferation, less apoptosis and amplified YAP is also able to induce EMT in cancer. When YAP is amplified the Hippo pathway cannot longer inhibit YAP and YAP is more present in the nucleus and forms a complex with TEAD. However in some cancers paradoxically YAP is down regulated and serves as a tumor suppressor. In tumors were YAP serves as oncogene, YAP could potentially be directly or indirectly targeted for anti-tumoral effect. In tumors were YAP serves as tumor suppressor YAP levels has to be indirectly restored. Hall et al. described that they observed that cancer cells were more sensitive for cisplatin if YAP was knocked down (24). This would mean that in cancers where YAP is up regulated, YAP inhibition could sensitize these cells for apoptosis.

As described before miRNA-375 plays an important role in the regulation of YAP. In HCC for example miRNA-375 is down regulated when YAP is up regulated. Liu et al found that if they brought miRNA-375 to expression in a HCC cell line that YAP expression decreased and mRNA levels of CTGF also decreased. A decrease of CTFG suggests that suppressing YAP by miRNA-375 may affect the YAP signaling output. They also observed that proliferation and invasion was significantly reduced in HCC cells when miRNA-375 was expressed. So miRNA-375 can serve as a potential therapeutic agent against YAP overexpression and prevent proliferation, invasion in HCC cells and perhaps make tumor cells more sensitive to cisplatin-induced apoptosis instead of less sensitive(26). Further research is needed to investigate possibly other targets of miRNA-375 to investigate its side-effects, and if miRNA-375 can be used as therapeutic agents in more cancer types where YAP is up regulated.

Camergo et al. found that Wnt and Notch signaling were altered in tumors where Yap is up regulated (9). They wanted to test if they inhibited Notch that the enlargement of the tissue would be

suppressed. To inhibit Notch they used γ-secretase inhibitors in mice during Yap activation. They found that during Yap activation and γ-secretase inhibitors the enlargement of the tissue was much less indicating a decrease in proliferation. These results do not rule out the possibility that γ-secretase inhibitors suppress Yap-induced enlargement by other biological processes other than Notch. However they are consistent with the model in which Yap-mediated enlargement of tissue at least relies partially on activation of the Notch signaling pathway(7). This potential therapy does not target YAP directly however this therapy can potentially be used to indirect target YAP in tumors were YAP is overexpressed. Whether tumors that express high levels of YAP are sensitive for γ-secretase inhibitors has to be further investigated.

In some cancers YAP serves as a tumor suppressor, Cottini et al. investigated what happened when YAP levels in those tumor cells are being restored to 'normal' levels. They discovered that YAP is mostly down regulated in hematologic malignancies, including lymphomas, leukemia's and multiple myelomas (27). They found that if they re-expressed YAP in multiple myelomas cell lines that it induced more apoptosis en reduce proliferation. YAP induced apoptosis was mediated by nuclear ABL1 activity, which is a proto-oncogene. When they treated the cells with both Imatinib, which is an ABL1 inhibitor, and YAP re-expression, apoptosis was significantly reduced. This suggests that YAP phosphorylation by ABL1 is required for apoptotic response. Because of the functional interaction between YAP and p73 they explored the relationship between YAP and p73 after DNA damage in multiple myeloma cells. Re-expression of YAP increased significantly p73 protein level and its transcriptional target genes such as BAX and PUMA. The results suggest that apoptosis in multiple myeloma cells induced by DNA damage and YAP re-expression is mediated by stabilization of p73 and increased expression of its downstream targets. MST1 normally interacts with Lats1/2, and reduces YAP levels. If they down regulated MST1 they saw that YAP levels restored and were comparable with the scrambled group. They saw that when YAP levels were restored with MST1 inhibition that also proliferation was reduced and induced an apoptotic response. They saw this phenomena not only in vitro but also in vivo, indicating that inhibition of MST1 in multiple myelomas who express low YAP could serve as an promising indirect therapeutic target to restore YAP levels (28).

Discussion

In this literature study an overview is provided of the function of Yap in the Hippo pathway and its role in tumorigenesis . Amplified YAP or down regulated YAP has been reported in many different tumor types so it is important to know the exact mechanism of YAP in tumorigenesis to understand the role of YAP in tumorigenesis. Interestingly YAP can serve as an oncogene but can also serve as tumor suppressor in the same but also in different tumor types (figure 2). Amplified YAP interacts with the transcription factor TEAD and activates CTGF which mediates proliferation, also amplified YAP can lead

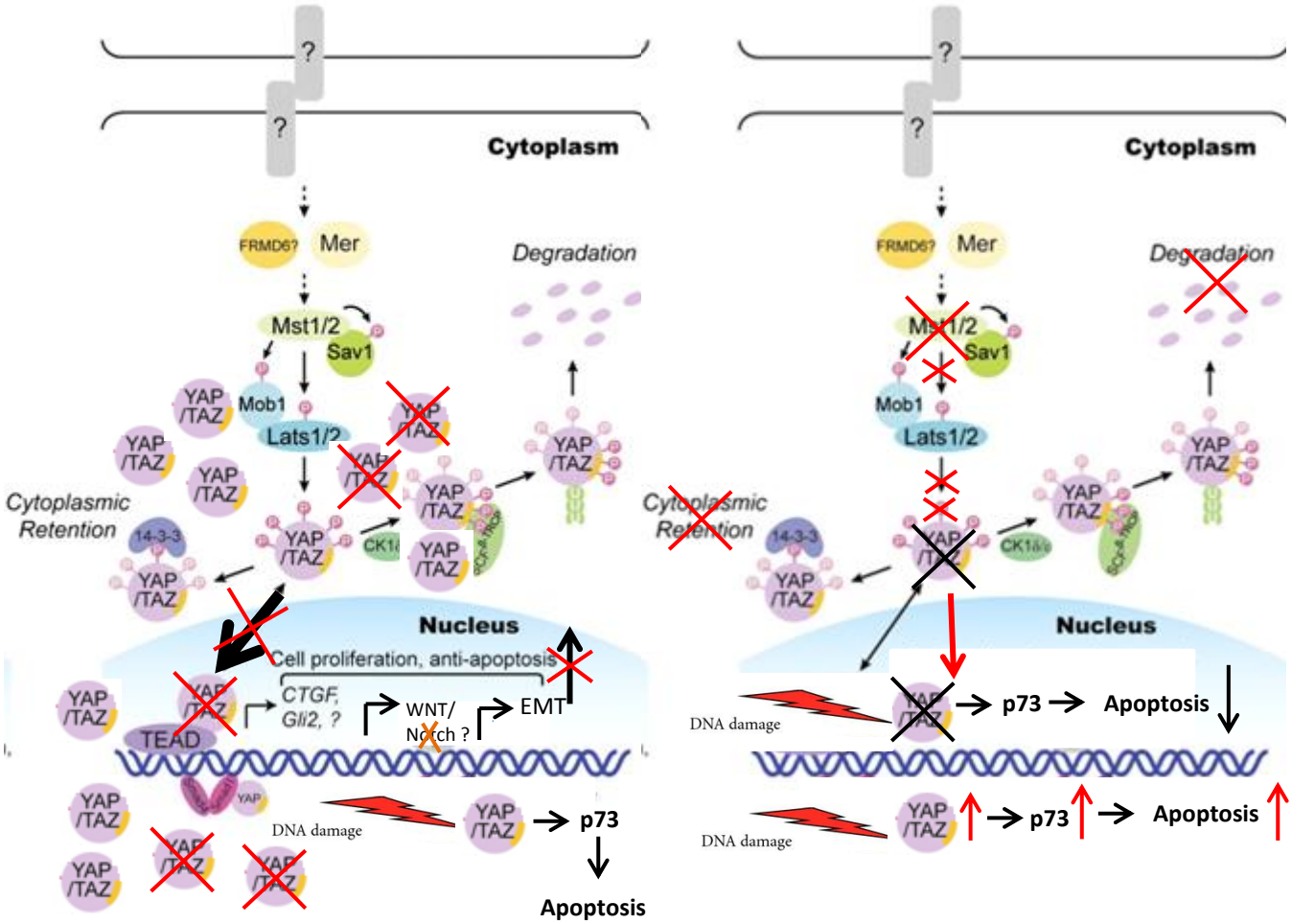

Figure 2: Oncogenic and tumor suppressor function of the Hippo-YAP pathway in tumorigenesis

A) Oncogenic function of the Hippo-YAP pathway is shown. As indicated by the black arrows amplified YAP has multiple effects: it can cause proliferation by activating CTGF, WNT/Notch signaling are altered, anti-apoptopic signals are increased and EMT occurs. There are two different possible therapies, one where YAP is directly targeted by re-expressing miRNA-375 as indicated by the red crosses. When YAP is directly targeted the excess of YAP is no longer present and increased proliferation and anti-apoptopic signals are being restored to normal levels. The second possible therapy is indirect targeting the increased Notch signaling by γ-secretase inhibitors in tumors where YAP is increased as indicated by the orange cross. This leads to a decreased enlargement indicating a decrease in proliferation.

B) Tumor suppressor function of the Hippo-pathway is shown. In some tumors Yap is down regulated as indicated by the black crosses, YAP cannot longer promote apoptosis by binding with p73 in case of DNA damage as indicated by the black arrow. Knockdown of YAP can result in suppressed anoikis, increased migration and invasiveness (data not shown). There is one possible therapy, inhibition of MST1/2 can indirectly re-express YAP because YAP can no longer be inhibited and ubiquitinated. Restored YAP levels lead to increased apoptosis and decreased proliferation.
Adapted by Zhao et al. (2010) (13).

to anti-apoptosis signals. Interestingly when YAP is amplified cells become less differentiated, a possible mechanism for this phenomena could be that YAP activates Wnt/Notch signaling. Amplified YAP can also lead to EMT in cells, which is considered important for tumorigenic progression (figure 2). Amplified Yap also has an important clinical relevance since high levels of YAP are associated with poor overall survival in patients with ovarian cancer and HCC. YAP can serve as new biomarker and therapy can be personalized if YAP expression is high since high expression of YAP is associated with a decreased sensitivity of cisplatin-induced apoptosis in ovarian cancer.

YAP can also serve as tumor suppressor gene when its down regulated. Down regulated YAP leads to suppressed anoikis, increased migration and invasiveness. Down regulated YAP cannot longer bind with p73 when DNA damage is present. Taken all this together can be concluded that the overexpression or down regulation of YAP in different tumor types can contribute to tumor development(figure 2).

When Yap is amplified or down regulated in certain cancer types it can serve as potential indirect or direct therapy target which leads to anti-tumoral effect. MiRNA-375 is found to serve as therapeutic agent against YAP which prevents proliferation and migration and makes tumor cells more sensitive to cisplatin-induced apoptosis. Also by targeting Notch signaling by γ-secretase inhibitors with YAP overexpression lead to decrease in proliferation. In tumors where YAP serves as a tumor suppressor inhibition of MST1 lead to an increase of YAP levels and a decrease of proliferation and increase in apoptosis (figure 2).

The rapid progress of the Hippo pathway research has provided a lot of information of the Hippo pathway and the role of YAP plays in tumorigenesis. YAP does not only serve as oncogene but can also serve as tumor suppressor gene. Interestingly YAP can serve as potential therapy for anti-tumoral effect target in both its function as oncogene and as tumor suppressor gene. The amount of YAP in the nucleus seems to play a very important role in the tumorigenesis, in some tumor types this is too low and in other tumor types this is too high indicating that a certain threshold of YAP in the nucleus seems to play an important role. The potential therapies are aimed to restore the levels of YAP to its normal levels. Potential therapy who target YAP which leads to anti-tumoral effect leads to new insights to prevents tumor growth where YAP plays an important role.

The picture of the Hippo pathway and the role of YAP in tumorigenesis is not complete and many questions remains to be addressed. YAP can induce anti-apoptotic signals but can also induce apoptosis by binding with a region of p73 in the presence of ABL1. How and when does YAP activate anti-apoptotic signals and when pro-apoptotic signals? Is this perhaps dictated by cell contexts? In nearly all the cases where YAP is amplified cIAP2 is also present, indicating a cooperation between YAP and cIAP2. However this phenomena has not yet been further investigated what their underlying relationship is. Also Wnt and Notch signaling is altered in tumors where YAP is amplified. These signaling pathways could be responsible for the expansion of the stem cells through activation of YAP, however it has to be further investigated how these signaling pathways are activated by YAP. It has to be investigated if YAP could serve in more cancer types as a prognostic marker and in tumors where YAP serves as an tumor suppressor does this also lead to a decreased overall survival? Also with the potential therapies a lot of research is still needed to answer questions such as can miRNA-375 be used as therapeutic target in vivo? And are tumors which express high levels of YAP sensitive for γ-secretase inhibitors. Also other potential targets such as TEAD has to be examined since TEAD is a direct

transcriptional cofactor of YAP. In this overview a lot of answers about YAP has been provided however a lot of questions still has to be answered.

References

1. Saucedo LJ, Edgar BA. Filling out the hippo pathway. Nature Reviews Molecular Cell Biology. 2007;8(8):613-21.

2. Potter CJ, Turenchalk GS, Xu T. < i> Drosophila</i> in cancer research: An expanding role. Trends in genetics. 2000;16(1):33-9.

3. Lamar JM, Stern P, Liu H, Schindler JW, Jiang ZG, Hynes RO. The hippo pathway target, YAP, promotes metastasis through its TEAD-interaction domain. Proc Natl Acad Sci U S A. 2012 Sep 11;109(37):E2441-50.

4. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, et al. Inactivation of YAP oncoprotein by the hippo pathway is involved in cell contact inhibition and tissue growth control. Genes Dev. 2007 Nov 1;21(21):2747-61.

5. Edgar BA. From cell structure to transcription: Hippo forges a new path. Cell. 2006;124(2):267-73.

6. Overholtzer M, Zhang J, Smolen GA, Muir B, Li W, Sgroi DC, et al. Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. Proc Natl Acad Sci U S A. 2006 Aug 15;103(33):12405-10.

7. Zender L, Spector MS, Xue W, Flemming P, Cordon-Cardo C, Silke J, et al. Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. Cell. 2006;125(7):1253-67.

8. Zhao B, Ye X, Yu J, Li L, Li W, Li S, et al. TEAD mediates YAP-dependent gene induction and growth control. Genes Dev. 2008 Jul 15;22(14):1962-71.

9. Camargo FD, Gokhale S, Johnnidis JB, Fu D, Bell GW, Jaenisch R, et al. YAP1 increases organ size and expands undifferentiated progenitor cells. Current biology. 2007;17(23):2054-60.

10. McClatchey AI, Giovannini M. Membrane organization and tumorigenesis--the NF2 tumor suppressor, merlin. Genes Dev. 2005 Oct 1;19(19):2265-77.

11. Bretscher A, Edwards K, Fehon RG. ERM proteins and merlin: Integrators at the cell cortex. Nature Reviews Molecular Cell Biology. 2002;3(8):586-99.

12. Hamaratoglu F, Willecke M, Kango-Singh M, Nolo R, Hyun E, Tao C, et al. The tumour-suppressor genes NF2/Merlin and expanded act through hippo signalling to regulate cell proliferation and apoptosis. Nat Cell Biol. 2006;8(1):27-36.

13. Willecke M, Hamaratoglu F, Kango-Singh M, Udan R, Chen C, Tao C, et al. The fat cadherin acts through the hippo tumor-suppressor pathway to regulate tissue size. Current biology. 2006;16(21):2090-100.

14. Zhao B, Li L, Lei Q, Guan KL. The hippo-YAP pathway in organ size control and tumorigenesis: An updated version. Genes Dev. 2010 May;24(9):862-74.

15. Pan D. The hippo signaling pathway in development and cancer. Developmental cell. 2010;19(4):491-505.

16. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. "Stemness": Transcriptional profiling of embryonic and adult stem cells. Science. 2002 Oct 18;298(5593):597-600.

17. Liu AM, Poon RT, Luk JM. MicroRNA-375 targets hippo-signaling effector YAP in liver cancer and inhibits tumor properties. Biochem Biophys Res Commun. 2010;394(3):623-7.

18. Strano S, Munarriz E, Rossi M, Castagnoli L, Shaul Y, Sacchi A, et al. Physical interaction with yes-associated protein enhances p73 transcriptional activity. J Biol Chem. 2001 May 4;276(18):15164-73.

19. Pan D. The hippo signaling pathway in development and cancer. Developmental cell. 2010;19(4):491-505.

20. Li Z, Chen J, Chan KW, Qiao L, Wong BC. A possible role of cIAP2 in< i> helicobacter pylori</i>-associated gastric cancer. Cancer Lett. 2011;313(2):192-200.

21. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009;139(5):871-90.

22. Ota M, Sasaki H. Mammalian tead proteins regulate cell proliferation and contact inhibition as transcriptional mediators of hippo signaling. Development. 2008 Dec;135(24):4059-69.

23. Yuan M, Tomlinson V, Lara R, Holliday D, Chelala C, Harada T, et al. Yes-associated protein (YAP) functions as a tumor suppressor in breast. Cell Death & Differentiation. 2008;15(11):1752-9.

24. Hall CA, Wang R, Miao J, Oliva E, Shen X, Wheeler T, et al. Hippo pathway effector yap is an ovarian cancer oncogene. Cancer Res. 2010 Nov 1;70(21):8517-25.

25. Xu MZ, Yao T, Lee NP, Ng IO, Chan Y, Zender L, et al. Yes-associated protein is an independent prognostic marker in hepatocellular carcinoma. Cancer. 2009;115(19):4576-85.

26. Cottini F, Hideshima T, Xu C, Sattler M, Dori M, Agnelli L, et al. Rescue of hippo coactivator YAP1 triggers DNA damage-induced apoptosis in hematological cancers. Nat Med. 2014.

27. Cottini F, Hideshima T, Xu C, Sattler M, Dori M, Agnelli L, et al. Rescue of hippo coactivator YAP1 triggers DNA damage-induced apoptosis in hematological cancers. Nat Med. 2014;20(6):599-606.