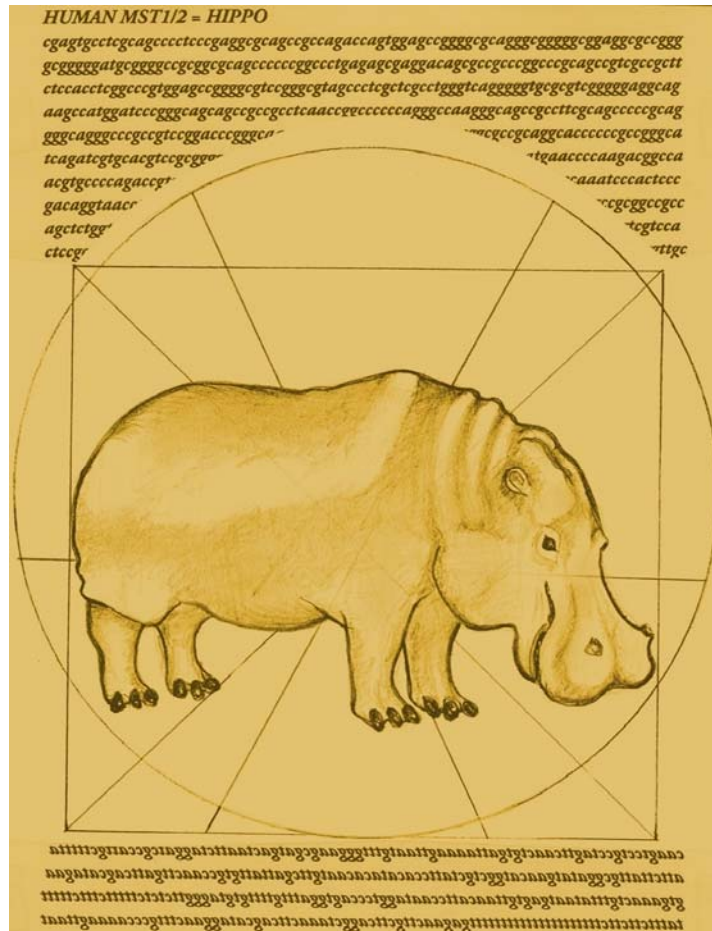


# The HIPPO Tumor Suppressor Pathway: A Brainstorming Workshop

Villa Betania, Rome 22-23 April 2009



Weizmann Institute of Science



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## Program and Abstracts

*Dear Colleagues,*

*On behalf of the Scientific Director, Prof.sa Paola Muti and the directors of the Regina Elena National Cancer Research Institute as well as the workshop organising committee, we would like to welcome you to the "brain storming" Hippo workshop at Villa Betania in Rome. This workshop is devoted to a newly emerging tumor suppressor pathway, the HIPPO pathway. We have assembled together leading researchers who represent two, seemingly opposing aspects of the pathway: the oncogenic signalling pathway and the apoptotic signalling pathway. The main purpose of the workshop over the next two days will be to identify common platforms and to plot new research directions. We intend to plan future international meetings, which should attract large and diverse groups of researchers.*

*On behalf of the Organising Committee we welcome you to Rome and we very much look forward to an exciting and invigorating two days.*

*The Organization Committee*

*Dr. Giovanni Blandino*

*Dr. Yosef Shaul*

*Dr. Marius Sudol*

*Dr. Michael Yaffe*

## **The HIPPO Tumor Suppressor Pathway: A Brainstorming Workshop**

*The aim of the gathering is to integrate the current knowledge of the Hippo pathway and to understand when the activation of this pathway results in proliferative and when in apoptotic signals*

### **Program**

**22 April 2009**

08:45 Welcome

Prof. Paola MUTI, IRE Scientific Director

S.E. Mons. Sergio PINTOR, President, Nicola's Foundation

09:00 - 10:00 Introduced and chaired by Dr. Giovanni Blandino

**Dr. Marius SUDOL** "From Src oncogene to WW domain: The role of YAP in the mammalian Hippo pathway"

**Dr. Mike YAFFE** "TAZ: A Mesenchymal Stem Cell Modulator of Cell Differentiation Moonlights as a Promoter of Tumor Cell Metastasis"

10:00 - 10:30 Short talk speakers,  
chaired by Dr. Marius Sudol & Dr. Mike Yaffe

**Dr. Kieran HARVEY** "Transcriptional output of the Salvador/Warts/Hippo pathway is controlled in distinct fashions in *Drosophila melanogaster*, and human breast epithelial cells"

**Dr. Iain FARRANCE** "Casein Kinase 2 (CK2) Interacts With and Regulates TEF-1 (TEAD1) DNA Binding and Transcriptional Activity"

10:30-11:00 Coffee Break

11:00 - 11:30 Introduced and chaired by Dr. Yosef Shaul

**by Dr. Georg HALDER** "Boundaries of Dachsous Cadherin activity modulate the Hippo signaling pathway to induce cell proliferation"

11:30-13:00 Short talk speakers,  
chaired by Dr. Georg Halder & Dr. Yosef Shaul

**Dr. Linda PARSONS** "Apico-basal polarity complexes regulate the Salvador/Warts/Hippo (SWH) pathway"

**Dr. Xinwei CAO** "The Hippo Pathway in Neural Development: Orchestrating the Proliferation, Survival, and Fate Choice of Neural Progenitor Cells"

**Dr. Nic TAPON** "The Hippo pathway regulates apico-basal polarity independently of its growth control function"

13:00-14:30 Lunch

14:30-16:00 "3 slide-mini-talks" Discussion, chaired by Dr. Marius Sudol & Dr. Mike Yaffe

16:00-16:30 Coffee Break

16:30 - 17:30 Introduced & chaired by  
Dr. Thijn Brummelkamp & Dr. Kun-Liang Guan

**Dr. Helen McNEILL** "Exploring the biochemical basis of growth control by Fat, Ex and Dco"

**Dr. Kun-Liang GUAN** "Regulation of the YAP and TAZ transcription co-activators by the Hippo tumor suppressor pathway"

17:30-18:30 Short talk speakers

**Dr. Hiroshi SASAKI** "The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophoctoderm from inner cell mass"

**Dr. Zafar NAWAZ** "Tumor suppressor, WWOX1, attenuates oncogenic YAP1 and WBP-2 mediated ER and PR transactivation functions."

### 23 April 2009

09:00-10:00 Introduced & chaired by

Dr. Eric O'Neill & Dr. Helen McNeill

**Dr. Giovanni BLANDINO** "YAP bridges p73 and PML pro-apoptotic pathways"

**Dr. Yosef SHAUL** "A Mechanism of Switching of Yap from pro-Oncogene to Potential Tumor Suppressor"

10:00-10:30 Short talk speakers

**Dr. Subham BASU** "JNK phosphorylates YAP to regulate stress signaling"

**Dr. David MATALANAS** "Regulation of the MST2 pathway by K-Ras"

10:30-11:00 Coffee Break

11:00-13:00 Short talk speakers, chaired by Dr. Kieran Harvey

**Dr. Yael AYLON** "The Lats2-Mdm2-p53 tumor suppressor axis"

**Dr. Elmar SCHIEBEL** "Function of the Hippo pathway at the human centrosome"

**Dr. Junichi SADOSHIMA** "Inhibition of Endogenous Lats2 Promotes Hypertrophy and Decreases Apoptosis in the Postnatal Heart"

13:00-15:00 Lunch

15:00 - 16:00 Introduced and chaired by Dr. Tobias Schmelzle & Dr. Rami Aqeilan

**Dr. Thijn BRUMMELKAMP** "How the size that tissues and organs reach during development is controlled"

**Dr. Hugo STOCKER** "New Signaling Partner of Expanded and Merlin".

**Dr. Eric O'NEILL** "RASSF1A/MST2 pathway and the DNA damage response"

16:00 Dr. Sabrina STRANO & Dr. Marius SUDOL

Concluding remarks and discussion panel

## **From Src oncogene to WW domain: The role of WW domains and PDZ-binding motif of YAP in the regulation of mammalian HIPPO pathway**

Marius Sudol<sup>1,2</sup>

<sup>1</sup>Weis Center for Research, Danville, PA 17822, USA

<sup>2</sup>Mount Sinai School of Medicine, Department of Medicine, New York, NY 10029, USA

YAP (Yes kinase-associated protein) is a WW domain-containing transcriptional co-activator that controls expression of genes regulating cell growth, proliferation and apoptosis. Both isoforms of YAP, YAP1 and YAP2, are effectors of the mammalian HIPPO pathway that controls organ size. YAPs have an ability to translocate from cytoplasm to nucleus. Under stress conditions of 1% serum, overexpression of YAP2 in cells causes cell detachment and apoptosis. We documented that YAP2 uses its WW domains to bind and stabilize pro-apoptotic member of the p53 family, p73, and that the YAP2-p73 complex promotes apoptosis.

YAP has also a PDZ-binding motif (-FLTWL) at its COOH terminus. As PDZ domains are frequently present in sub-membrane located scaffold proteins, we wanted to examine if YAP2 localization in cells is PDZ dependent. We generated delta C mutant of YAP2 lacking five most COOH terminal amino acids, which constitute a well-conserved PDZ-binding motif. We found that the PDZ-binding motif was necessary for YAP2 localization in the nucleus. YAP delta C bound p73 but did not stabilize it. YAP delta C did not promote apoptosis of cells grown in low serum. We suggest that an unknown PDZ domain-containing protein(s) functions as a shuttle facilitating YAP2 translocation from the cytoplasm to the nucleus. As fly YAP, yki does not have a PDZ-binding motif, we indicate that this mechanism may not be conserved in *Drosophila*. Since the Hippo pathway is a tumor suppressor pathway, the PDZ complex of human YAP represents a potential target of cancer therapy

## **TAZ: A Mesenchymal Stem Cell Modulator of Cell Differentiation Moonlights as a Promoter of Tumor Cell Metastasis.**

Jeong-Ho Hong, H. Christian Reinhardt, Pia Hasskamp, Daniel Lim, Eun Sook Hwang and Michael B. Yaffe  
Koch Institute for Integrative Cancer Biology, MIT, Boston, MA USA

TAZ is a paralog of the tumor suppressor gene YAP, that plays an important role in regulating mesenchymal stem cell (MSC) fate. TAZ interacts through its WW domain with key transcription factors involved in MSC differentiation, including Runx2 and PPAR $\gamma$ . TAZ binding to Runx2 promotes bone, muscle and cartilage differentiation, while TAZ binding to PPAR $\gamma$  suppresses adipocyte differentiation. I will present new data indicating that TAZ is coordinately regulated by the kinases Lats2 and GSK3 $\beta$ , targeting it for ubiquitin mediated proteolysis through binding to  $\beta$ -TrCP. Mutations which prevent TAZ phosphorylation within a non-canonical phosphodegron result in elevated TAZ levels in cells, and enhanced metastatic behavior when these mutant TAZ-expressing cells are injected into murine tumor model systems in vivo. No such phosphodegron is found within YAP. Our data suggests that TAZ, but not YAP, is a GSK3 $\beta$ -regulated promoter of tumor metastasis.

# **Transcriptional output of the Salvador/Warts/Hippo pathway is controlled in distinct fashions in *Drosophila melanogaster*, and human breast epithelial cells**

Xiaomeng Zhang<sup>1,3</sup>, Claire C. Milton<sup>1,3</sup>, Patrick O. Humbert<sup>2,3</sup> and Kieran F. Harvey<sup>1,3\*</sup>

<sup>1</sup> Cell Growth and Proliferation Laboratory, and

<sup>2</sup> Cell Cycle and Cancer Genetics Laboratory, Peter MacCallum Cancer Centre, 7 St Andrews Place, East Melbourne, Victoria, Australia, 3002

<sup>3</sup> Department of Pathology, University of Melbourne, Parkville, Victoria, Australia, 3010

The Salvador-Warts-Hippo (SWH) pathway is an important modulator of organ size and deregulation of pathway activity can lead to cancer. The SWH pathway regulates tissue growth by restricting activity of the transcriptional co-activator protein known as Yorkie in *Drosophila melanogaster*, and YAP in mammals. Yorkie/YAP drives tissue growth in partnership with the Scalloped/TEAD1-4 transcription factors. Yorkie/YAP also possesses two WW domains, which contact several proteins that have been suggested to either promote or inhibit Yorkie's ability to induce gene transcription. To investigate the regulatory role of the WW domains of Yorkie/YAP in detail we analysed the effect of mutating these domains on Yorkie/YAP's function. WW domain mutant YAP promoted transformation and migration of breast epithelial cells with increased potency, suggesting that WW domains mediate inhibitory regulation of YAP in these cells. By contrast, the WW domains were required for Yorkie's ability to promote tissue growth in *Drosophila melanogaster* and to optimally activate the Scalloped transcription factor. These results demonstrate that Yorkie/YAP WW domains have distinct regulatory roles in different cell types, and imply the existence of proteins that promote tissue growth in collaboration with Yorkie and Scalloped.

## **Casein Kinase 2 (CK2) Interacts With and Regulates TEF-1 (TEAD1) DNA Binding and Transcriptional Activity**

Mahoney, WM, Francis, MF, Lafferty, MK and Farrance, IK. Department, of Biochemistry, University of Maryland, Baltimore, MD 21201 USA

TEF-1 (TEAD) family members are key transcriptional regulators in the heart during development, under normal physiological conditions, and during the hypertrophic response. TEF-1 requires cofactors for activity, that include p160, vestigial-like proteins (Vgl-1 to -4), PARP, and YAP1 and TAZ. We undertook studies to identify additional TEF-1 cofactors active in cardiac transcription. A yeast 2-hybrid screen with aa 111-430 of rat TEF-1 isolated the regulatory subunit of Casein Kinase 2 (CK2beta). CK2 is a ubiquitous ser/thr protein kinase consisting of two regulatory beta and two catalytic alpha subunits whose activity is regulated by targeting CK2 holoenzyme or CK2beta containing complexes to intracellular locations.

Here we show that TEF-1 interacts with CK2beta *in vitro*, by GST-pulldown assays, and *in vivo*, by co-IP assays. Furthermore, TEF-1 interacts the active CK2 holoenzyme *in vivo*. TEF-1 is phosphorylated near the amino terminus in cardiac and skeletal muscle, but how TEF-1 phosphorylation affects TEF-1 activity was not known. CK2 phosphorylates TEF-1 *in vitro* and phosphorylation of TEF-1 at S11 by CK2 inhibits the binding of TEF-1 to DNA. CK2 also regulates TEF-1 activity *in vivo* as a CK2 inhibitor activates TEF-1-dependent promoters.

We propose that the TEF-1:CK2 association may regulate transcription by three mechanisms: (1) CK2 phosphorylation of TEF-1 inhibits TEF-1 binding to promoter regulatory elements; (2) TEF-1 recruits CK2beta and associated proteins to DNA, regulating the activity of nearby transcription factors or chromatin structure; and (3) TEF-1 recruits CK2 holoenzyme to muscle promoters, where CK2 can regulate the activity of other substrates.

## **Boundaries of Dachsous Cadherin activity modulate the Hippo signaling pathway to induce cell proliferation**

Maria Willecke, Fisun Hamaratoglu, Leticia Sansores-Garcia, Chunyao Tao, and Georg Halder, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, USA

The conserved Hippo tumor suppressor pathway is a key signaling pathway that controls organ size in *Drosophila*. To date a signal transduction cascade from the Cadherin Fat at the plasma membrane into the nucleus has been discovered. However, how the Hippo pathway is regulated by extracellular signals is poorly understood. Fat not only regulates growth but also planar cell polarity, for which it interacts with the Dachsous (Ds) Cadherin, and Four-jointed (Fj), a transmembrane protein that modulates the interaction between Ds and Fat. Ds and Fj are expressed in gradients and manipulation of their expression causes abnormal growth. However, how Ds and Fj regulate growth and whether they act through the Hippo pathway is not known. We found that Ds and Fj regulate Hippo signaling to control growth. Interestingly, we found that Ds/Fj regulate the Hippo pathway through a remarkable logic. Induction of Hippo target genes is not proportional to the amount of Ds or Fj presented to a cell, as would be expected if Ds and Fj acted as traditional ligands. Rather, Hippo target genes are upregulated when neighboring cells express different amounts of Ds or Fj. Consistent with a model that differences in Ds/Fj levels between cells regulate the Hippo pathway, we found that artificial Ds/Fj boundaries induce extra cell proliferation, while flattening the endogenous Ds and Fj gradients results in growth defects. The Ds/Fj signaling system thus defines a cell-to-cell signaling mechanism that regulates the Hippo pathway thereby contributing to the control of organ size.

## **Apico-basal polarity complexes regulate the Salvador/Warts/Hippo (SWH) pathway.**

<sup>1</sup>Linda M. Parsons, <sup>1</sup>Nicola A. Grzeschik, <sup>4</sup>Melinda L. Allott, <sup>2</sup>Kieran F. Harvey and <sup>1</sup>Helena E. Richardson

<sup>1</sup>Peter MacCallum Cancer Centre, East Melbourne, Vic., Australia

<sup>2</sup>Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley

<sup>4</sup>Department of Veterinary Science, The University of Melbourne, Parkville, Victoria, Australia

In *Drosophila*, it has long been recognized that loss-of-function mutations in the junctional neoplastic tumour suppressors, Lethal-2-giant-larvae (Lgl), Dlg and Scribble disrupt apico-basal polarity in epithelial tissues and induce over-proliferation. Here, for the first time, we provide evidence for a connection between Lgl and the Salvador/Warts/Hippo (SWH) pathway in tissue growth regulation. We have shown that in the developing *Drosophila* eye, loss of *lgl* activity results in ectopic proliferation and suppression of developmental cell death (apoptosis), without loss of cell polarity. In this system, targets of the SWH pathway are increased upon Lgl downregulation. This occurs by decreased phosphorylation and increased nuclear localization of the SWH pathway coactivator, Yorkie (Yki). Moreover, our genetic evidence shows that Yki is rate limiting for the development of *lgl* phenotypes. Conversely, downregulation of Dlg and Scrib, to levels where cell polarity is not affected, does not lead to increased Yki activity. This separates the role of Lgl, from that of Dlg and Scrib, in SWH pathway regulation in the eye epithelium. Consistent with the antagonistic relationship between Lgl and the apical cell polarity regulators, Crumbs and, aPKC, overexpression of Crumbs and aPKC also lead to the upregulation of SWH pathway targets. Taken together, our genetic and biochemical data reveals that Lgl acts antagonistically to Crumbs and aPKC to regulate SWH signaling and provides evidence for a novel pathway connecting apico-basal cell polarity to the SWH pathway in the regulation of tissue growth.

## **The Hippo Pathway in Neural Development: Orchestrating the Proliferation, Survival, and Fate Choice of Neural Progenitor Cells**

Xinwei Cao<sup>1</sup>, Samuel L. Pfaff<sup>2,4</sup>, and Fred H. Gage<sup>1,3</sup>

<sup>1</sup>Laboratory of Genetics, <sup>2</sup>Howard Hughes Medical Institute, Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA

Tight control of cell proliferation is essential for proper growth during development and for tissue homeostasis in mature animals. The evolutionarily conserved Hippo pathway restrains proliferation through a kinase cascade that culminates in the inhibition of the transcriptional coactivator YAP. Unphosphorylated YAP activates genes involved in cell proliferation and survival by interacting with a DNA-binding factor. We find that during vertebrate neural tube development, the TEA-domain transcription factor (TEAD) is the cognate DNA-binding partner of YAP. YAP and TEAD gain-of-function causes marked expansion of the neural progenitor population, partly owing to their ability to promote cell-cycle progression by inducing *cyclin D1* and to inhibit differentiation by suppressing *NeuroM*. Their loss-of-function results in increased apoptosis, whereas repressing their target genes leads to premature neuronal differentiation. Inhibiting the upstream kinases of the Hippo pathway also causes neural progenitor overproliferation. Thus, the Hippo pathway plays critical roles in regulating neural progenitor cell number by affecting proliferation, fate choice, and cell survival.

## **The Hippo pathway regulates apico-basal polarity independently of its growth control function**

A. Genevet<sup>1</sup>, C. Polesello<sup>1,2</sup>, F. Robertson<sup>3</sup>, F. Pichaud<sup>3</sup>, K. Blight<sup>4</sup>, L. Collinson<sup>4</sup>, N. Tapon<sup>1</sup>. 1) Apoptosis and Proliferation Control Laboratory, Cancer Research UK, London Research Institute, London, United Kingdom; 2) Centre de Biologie du Développement, UMR5547, CNRS/Université Paul Sabatier Toulouse III, Toulouse, France; 3) MRC Laboratory for Molecular Cell Biology and Cell Biology Unit, Department of Anatomy and Developmental Biology, University College London, London, United Kingdom; 4) Electron Microscopy Unit, Cancer Research UK, London Research Institute, London, United Kingdom.

The Hippo pathway, identified in *Drosophila* and conserved in vertebrates, regulates tissue growth by promoting cell cycle exit and apoptosis. In addition to their well characterised overproliferation phenotype, epithelial cells mutant for the kinases Hippo and Warts present a hypertrophy of the apical domain. We examined the molecular basis of this apical hypertrophy and its impact on cell proliferation. In the wing imaginal disc epithelium, we observe increased staining for the apical polarity complexes, such as DaPKC/Par3/Par6 and Crumbs/Stardust when Hippo activity is compromised, while baso-lateral markers are not affected. The cell surface localisation of the Notch receptor is also increased in mutant clones, opening the possibility that aberrant receptor signalling may participate in overgrowth of hpo-deficient tissue. Interestingly however, while the polarity determinant Crumbs is required for the accumulation of apical proteins, this does not appear to significantly contribute to the overproliferation defect elicited by loss of Hippo signalling. Therefore, Hippo signalling controls polarity and growth via distinct mechanisms.

## **Exploring the biochemical basis of growth control by Fat, Ex and Dco.**

Caroline Badouel, Richelle Sopko and Helen McNeill

Samuel Lunenfeld Research Institute, Toronto, Ontario M5G 1X5, Canada

A major outstanding question in growth control is how the Hippo pathway is regulated during normal development. Work from a number of laboratories has implicated the large cadherins Fat and Dachshous, the FERM domain proteins Expanded and Merlin, and the kinase Discs overgrown (Dco) in regulation of growth by the Hippo pathway.

However the biochemical details of how these proteins regulate growth are still unclear. We will present our recent progress exploring the interactions of 1) Fat and Dco, 2) Fat and Expanded and 3) Expanded and Yorkie in the regulation of growth in *Drosophila*. These data lead to a model for growth regulation subsequent to Dachshous binding to Fat in vivo.

## **Regulation of the YAP and TAZ transcription co-activators by the Hippo tumor suppressor pathway**

Kun-Liang Guan - Department of Pharmacology and Moores Cancer Center  
University of California at San Diego, La Jolla, CA 92093

The YAP is a transcription co-activator and a candidate human oncogene. YAP is amplified in some human cancers and plays a key role in organ size regulation. We showed that YAP is phosphorylated and inhibited by the Hippo tumor suppressor pathway. Phosphorylation of YAP by the Lats tumor suppressor kinase results in 14-3-3 binding and cytoplasmic localization, therefore inactivating YAP. The nuclear-cytoplasmic shuttling of YAP is regulated by cell density, suggesting a role of YAP in cell contact inhibition. Furthermore, we have established that the TEAD family transcription factors play a key role in mediating the biological functions of YAP. TEAD is required for YAP to induce gene expression and promote cell proliferation. Moreover, we have also shown that the WW domain of YAP has a critical role in inducing a subset of YAP target genes independent of or in cooperation with TEAD. Mutation of the WW domains diminishes the ability of YAP to stimulate cell proliferation and oncogenic transformation. TAZ is a transcription co-activator closely related to YAP and is regulated by the Hippo pathway in a manner similar to YAP. Together, our studies establish a molecular mechanism of YAP regulation by the Hippo tumor suppressor pathway.

## **The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass**

Hiroshi Sasaki (RIKEN Center for Developmental Biology, Kobe, Japan)

Outside cells of the preimplantation mouse embryo form the trophectoderm (TE). We previously showed that TE development requires the transcription factor Tead4. However, the mechanism, by which widely expressing Tead4 promotes TE development only in the outer cells, remains elusive. Here, we show that increase in transcriptional activity of Tead4 is sufficient to induce *Cdx2* and other trophoblast genes in parallel in embryonic stem cells. In embryos, the Tead4 coactivator protein Yap localizes to nuclei of outside cells, and modulation of Tead4 or Yap activity leads to changes in *Cdx2* expression. In inside cells, Yap is phosphorylated and cytoplasmic, and this involves the Hippo signaling pathway component Lats. Inhibition of nuclear localization of Yap in the inner cells also requires cell-cell contacts. We propose that active Tead4 promotes TE development in outside cells, while Tead4 activity is suppressed in inside cells by cell contact- and Lats-mediated inhibition of nuclear Yap localization. Thus differential signaling between inside and outside cell populations leads to changes in cell fate specification during TE formation.

## **Tumor suppressor, WWOX1, attenuates oncogenic YAP1 and WBP-2 mediated ER and PR transactivation functions.**

Sarath C. Dhananjayan<sup>1</sup>, Rami I. Aqeilan<sup>2</sup>, and Zafar Nawaz<sup>1</sup>.

<sup>1</sup>Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, FL, USA; <sup>2</sup>The Hebrew University-Hadassah Medical School, The Lautenberg Center for Immunology, Jerusalem 91120, Israel.

We cloned WW-domain binding protein-2 (WBP-2), a PY motif containing protein as a selectively coactivator of ER and PR transactivation functions. PY motifs specifically interact with the WW domains of proteins like yes associated protein (YAP1) and WW-domain-containing oxidoreductase (WWOX1). In this study we investigate the molecular mechanism of action of WBP-2 in ER and PR signaling. We show that YAP1 and WWOX1 interact with the PY3 motif of WBP-2. We also show that YAP1 acts as a secondary coactivator of ER and PR, which is revealed only in the presence of wild-type WBP-2. Furthermore, YAP1 and WBP-2 are recruited to the ER responsive pS2 and GREB1 promoters and they synergistically enhance the transactivation functions of ER and PR. This synergistic increase is abrogated by WWOX1. Recruitment of WBP-2 and YAP1 to ER-responsive promoters is strictly inter-dependent. This suggests that the interaction between WBP-2 and YAP1 is critical of the coactivation functions of both the proteins. Based on our data, we propose a mechanistic model that the coactivation functions of oncogenic YAP1 is dependent on WBP-2 and that WWOX1 acts as a tumor suppressor by interacting with WBP-2 and retaining it in the cytoplasm, resulting in the attenuation of WBP-2 and oncogenic YAP1 mediated ER and PR transcriptional activities. This complex regulatory mechanism is dependent on the PY-WW interaction module, which represents the first instance, where a tumor suppressor (WWOX1) has been shown to inhibit the transcription activation functions of steroid hormone receptor coactivators (WBP-2 and YAP1).

## **YAP bridges p73 and PML pro-apoptotic pathways**

Lapi E<sup>1</sup>, DiAgostino S.<sup>2</sup>, Donzelli S.<sup>2</sup>, Fausti F.<sup>2</sup>, Bertini E.<sup>2</sup>, Lu X.<sup>1</sup>, Strano S.<sup>2</sup>, Blandino G.<sup>2</sup>.

<sup>1</sup>Ludwig Cancer Institute, Oxford University, UK <sup>2</sup>Regina Elena Cancer Institute, Rome, Italy.

p73 has been identified as a structural and functional homologue of the tumour suppressor p53. The transcriptional coactivator Yes-associated protein (YAP) has been demonstrated to interact with and to enhance p73-dependent apoptosis in response to DNA damage. YAP contributes to p73 stabilization in response to DNA damage and promotes p73-dependent apoptosis through the specific and selective coactivation of apoptotic p73 target genes and potentiation of p300-mediated acetylation of p73.

The PML tumour suppressor gene, involved in the t(15;17) chromosomal translocation of acute promyelocytic leukemia (APL), encodes a protein that localizes to the PML-nuclear body. PML has been shown to be involved in apoptosis: it is markedly upregulated upon a number of cellular stresses including inflammation, oncogenic transformation and proapoptotic stimuli such as ionizing radiation.

Here we show the existence of a pro-apoptotic auto-regulatory feedback loop between p73, YAP and PML during apoptosis triggered by CDDP in HCT116 cells. It has been previously demonstrated that YAP requires PML and NBs localization to coactivate p73. Here we show that p73 and YAP are required for the transcriptional activation of PML during the apoptotic response and for the subsequent accumulation of PML protein and formation of nuclear bodies. As a consequence, PML can contribute to the p73-dependent apoptotic response by promoting both p300-mediated acetylation of p73 and stabilizing YAP by inhibiting its ubiquitin-mediated degradation.

Hence, we determine a mechanistic pathway in response to DNA damage that could have relevant implications for the treatment of human cancer.

## **A Mechanism of Switching of Yap from pro-Oncogene to Potential Tumor Suppressor**

Nina Reuven, Dan Levy and Yosef Shaul. Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 76100, ISRAEL

It appears that a number of proteins act as double-edged sword regulators to behave as oncogenes under one condition while adopting the role of tumor suppressor under other conditions. To study the molecular basis of this switching process we investigated the transcription coactivator Yap1, which has been shown to coactivate the tumor suppressor p73 in the induction of apoptosis, but which has also been shown to have pro-proliferative, anti-apoptotic activities in other settings. Yap1 the downstream target of Hippo signaling, determines organ size in species from *Drosophila* to mammals, by inducing the expression of a number of anti-apoptotic genes. We found that Yap1 coactivates Runx in inducing Itch. Itch is an E3 ligase responsible for p73 degradation. Thus, Yap1 not only activates anti-apoptotic genes but also destroys an activator of pro-apoptotic genes. It has been shown that over-expression of Yap1 in liver gives rise to tumors. In sharp contrast, in response to DNA damage, Yap1 in collaboration with p73 activates pro-apoptotic genes. This process depends on the tyrosine kinase c-Abl. We found that in response to DNA damage c-Abl is activated and tyrosine phosphorylates both p73 and its coactivator Yap1. We further found that the tyrosine phosphorylation of Yap1 changes the repertoire of its target genes. Naïve Yap1 coactivates the Runx transcription factor to downregulate p73, whereas the modified Yap1 dissociates from Runx to support p73 accumulation. Furthermore, the modified Yap1 becomes engaged with p73 to support the expression of the pro-apoptotic genes. This is a mechanistic demonstration of how a coactivator changes its targets in response to the nature of the upstream signaling, from anti-apoptotic to pro-apoptotic genes with implications in oncogenesis.

## **JNK phosphorylates YAP to regulate stress signaling**

Katrin Gudmundsdottir<sup>1§</sup>, Victoria Tomlinson<sup>1§</sup>, Phuong Luong<sup>1</sup>, Axel Knebel<sup>2</sup> and Subham Basu<sup>1</sup>

<sup>1</sup>Cell Survival Signalling Laboratory, Centre for Molecular Oncology and Imaging, Institute of Cancer, Barts and the London School of Medicine, Queen Mary University of London, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom;<sup>2</sup>Kinasource Ltd, Unit 9 South Dudhope Mill, 77 Douglas Street, Dundee DD1 5AN, UK

<sup>§</sup>These authors contributed equally to this work.

YAP functions as a transcriptional co-factor that modulates the expression of genes involved in cell growth, proliferation and apoptosis. Upon DNA damage, YAP binds and stabilizes p73 to promote its expression of pro-apoptotic genes. In contrast to this putative tumour-suppressive role, YAP has also binds TEAD transcription factors to up-regulate pro-growth genes. Differences in upstream signalling may explain the apparently contradictory roles of YAP. Akt has been shown to block YAP's pro-apoptotic role via p73 binding, countered by c-Abl activation, whereas LATS1 phosphorylation of YAP has been shown to both promote p73 binding and block TEAD co-activation.

The aim of this project was to gain further understanding of the role of YAP in the cell by discovering new pathways affecting YAP function. Through a kinase screen we identified JNK1, 2 as kinases that robustly phosphorylate YAP *in vitro* and mapped phosphorylation sites by mass spectrometry. We generated both JNK phospho-site YAP mutant constructs and a JNK phospho-specific YAP antibody. Employing anisomycin and UV radiation as well as specific kinase inhibitors, we demonstrated JNK phosphorylation of YAP in a number of cell types. In contrast to what is reported for phosphorylation by c-Abl upon cisplatin treatment, UV stimulation of JNK phosphorylation resulted in YAP protein degradation. Silencing of YAP or expression of JNK phospho-site YAP mutants enhanced UV induced apoptosis, implicating JNK phosphorylation of YAP to be anti-apoptotic. We also detect JNK phosphorylation of YAP in cisplatin treated cells, indicating that YAP integrates multiple pathways to determine eventual apoptotic output.

## **Regulation of the MST2 pathway by K-Ras**

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The mammalian MST2 pathway has been implicated in the control of organ size and apoptosis. We have recently shown that the pro-apoptotic activity of MST2 is regulated by the proto-oncogen Raf-1 and the tumour suppressor RASSF1A. The activation of MST2 upon RASSF1A interaction ultimately regulates p73 transcriptional activity through regulation of LATS1 and YAP1 tumour suppressors resulting in apoptosis. To maintain tissue homeostasis pro-apoptotic pathways must be coordinated with proliferation signals. The pro-apoptotic effect of K-Ras has been known for several years but is poorly understood. As with other oncogenes, mutant Ras has also been suggested to induce an apoptotic response co-ordinately with its mitogenic role. It has been proposed before that RASSF1A-MST2 pro-apoptotic signaling may be regulated by K-Ras. Here we present evidence that demonstrate dual regulation of the MST2 pathway by K-Ras. In the presence of growth factor, K-Ras binds to the RASSF1A-MST2 complex, but prevents the activation of apoptosis. However, upon serum deprivation activated K-Ras also interacts with RASSF1A-MST2 complex but increasing its pro-apoptotic signal through activation of LATS1. Our results demonstrate that MST2 pathway plays a major role in K-Ras regulation of apoptosis.

## **The Lats2-Mdm2-p53 tumor suppressor axis**

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Damage to the mitotic spindle and centrosome dysfunction can lead to cancer. To prevent this, cells trigger a succession of checkpoint responses, where an initial mitotic delay is followed by slippage without cytokinesis, spawning tetraploid G1 cells that undergo a p53-dependent G1/S arrest. We have previously characterized the importance of Lats2 (mammalian ortholog of Warts) in this checkpoint response. Specifically, the Lats2-p53 axis is critical for the maintenance of proper chromosome number in the face of mitotic insults. Several oncogenic and tumor suppressor pathways impinge on and modulate the activity of this axis. For instance, depletion of the tumor suppressor and ubiquitin ligase Fbw7 limits the ability of Lats2-p53 to respond to mitotic toxins. In our current work, we demonstrate that the oncogenic form of HRas initially hyperactivates the Lats2-p53 checkpoint. However, cells surviving sustained oncogenic HRas expression actually neutralize the Lats2-p53 tumor suppressor pathway and emerge with features of transformation, such as genomic instability. Our data suggest that restraining the activity of this pathway might be an important step in cell transformation and tumor progression.

## **Function of the Hippo pathway at the human centrosome**

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The Hippo pathway has a well-established function in tissue growth control, apoptosis and in the regulation of cytokinesis. It has also been demonstrated that many of the Hippo pathway components localize to centrosomes. However, the function of Hippo pathway at this location is unclear. Here, we showed that in mammalian cells the scaffold protein hSav1 localizes to the centrosomes throughout the cell cycle and to the midbody during late telophase/cytokinesis. We analysed the binding requirements of hSav1 to the centrosomes and found that a previously uncharacterized region is responsible for centrosomal binding. In order to find interaction partners for hSav1 on the centrosomes, we performed a yeast-two-hybrid screen which identified important regulators of mitosis as interaction partners of hSav1 and in addition the PAK kinases Mst1/2, two known interactors of hSav1. Further analysis showed that hSav1 directly interacts with these cell cycle regulators and that they become phosphorylated by Hippo pathway kinases. Identification and analysis of the phosphorylation sites in the target proteins and RNAi depletion experiments allowed us to unravel the molecular function of the Hippo pathway at the centrosomes. Thus, this work describes a new and unexpected role of the Hippo pathway at the mammalian centrosome.

## **Inhibition of Endogenous Lats2 Promotes Hypertrophy and Decreases Apoptosis in the Postnatal Heart**

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Lats2, a component of the Hippo pathway, plays an important role in regulating cell proliferation and cell death in mammalian cells. We have shown previously that overexpression of Lats2 inhibits hypertrophy and promotes apoptosis in response to pressure overload in the mouse heart. However, the function of endogenous Lats2 in the heart is not fully understood. Although homozygous disruption of the Lats2 gene causes embryonic lethality in mice, Lats2<sup>+/-</sup> mice are born at the expected Mendelian ratio. Lats2<sup>+/-</sup> mice exhibited cardiac hypertrophy compared to wild type (WT) mice at 8 months of age (heart weight/body weight 4.48 and 4.21,  $p < 0.01$  vs WT,  $n=4$ ). Heterozygous deletion of Lats2 induced significant enlargement of the myocyte cross sectional area (333 and 288  $\mu\text{m}^2$ ,  $p < 0.05$  vs WT,  $n=6$ ). This hypertrophy could be compensatory since Lats2<sup>+/-</sup> mice also have fewer myocytes in the heart ( $9.7 \times 10^6$  and  $1.2 \times 10^7$ ,  $p < 0.05$  vs WT,  $n=4$ ). Despite the enlargement of the heart, histological analyses showed that Lats2<sup>+/-</sup> mice have a significantly smaller number of TUNEL positive cells (0.04 and 0.09%,  $p < 0.05$  vs WT,  $n=4$ ) and less fibrosis compared to WT mice (0.9 and 1.6%,  $p < 0.05$  vs WT,  $n=4$ ). Echocardiographic measurements indicated that Lats2<sup>+/-</sup> mice have better baseline left ventricular systolic function than WT mice at 5 months of age (ejection fraction 70 and 60%,  $n=4$ ). These results suggest that endogenous Lats2 negatively regulates the size of cardiac myocytes and stimulates apoptosis, thereby mimicking the function of Wts, the *Drosophila* counterpart of mammalian Lats2.

## **How the size that tissues and organs reach during development is controlled**

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How the size that tissues and organs reach during development is controlled is poorly understood. The barrier that normal tissues encounter when they have reached their correct size may impact on the very early stages of tumorigenesis. A recently discovered signaling pathway, named the Hippo signaling pathway, regulates organ size in *Drosophila* as well as in mammals. Various components of this signaling pathway are deregulated or mutated in human cancer. It is unclear whether the pathways that control organ size also impinge on stem/progenitor cells. A highly expressed gene in stem cells is YAP1, the ortholog of *Drosophila* Yorkie, a downstream component of the Hippo pathway. Activation of YAP1 in mice increases organ size and causes aberrant tissue expansion in mice. YAP1 activation reversibly increases liver size more than 4-fold. In the intestine, expression of endogenous YAP1 is restricted to the progenitor/stem cell compartment, and activation of YAP1 expands multipotent undifferentiated progenitor cells, which differentiate upon cessation of YAP1 expression. YAP1 stimulates Notch signaling, and administration of gamma-secretase inhibitors suppressed the intestinal dysplasia caused by YAP1. Human colorectal cancers expressing higher levels of YAP1 share molecular aspects with YAP1-induced dysplastic growth in the mouse. This suggests a potential link between stem/progenitor cells, organ size, and cancer.

## **RASSF1A/MST2 pathway and the DNA damage response.**

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The mammalian sterile20 like kinase MST2, and its drosophila homolog hippo, have been implicated in the control of apoptosis and the cell cycle through activation of a number of downstream substrates. We have previously shown that the pro-apoptotic activity of MST2 is under the control of the Raf-1 kinase and signalling through RAS. The inhibitory effects of Raf-1 are alleviated by the tumour suppressor RASSF1A which triggers a signaling cascade through LATS and YAP leading to p73 proapoptotic transcription. Recently, we have begun to address how the DNA damage response acting through YAP and p73 is regulated by RASSF1A and can confirm that the pathway is required for a normal apoptotic response to DNA damage. Moreover, ATM driven phosphorylation of RASSF1A is required for suppression of colony formation in cells exposed to radiation or chemotherapeutic agents. Taking this information together with clinical observations that methylation of genes including RASSF1A appear to ablate therapeutic responses, the DNA damage response is likely to be a substantial contributor to the tumour suppressor effects of RASSF1A.

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## **Interaction with TEAD proteins is important for TAZ to transform mammary epithelial MCF10A cells**

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The DSGXXS phosphodegron dictates regulated phosphorylation, ubiquitination, and proteasome-mediated degradation of  $\beta$ -catenin, I $\kappa$ B and an increasing number of other proteins. Database searches revealed the existence of over 100 proteins harboring the degron. Pilot analysis of 6 proteins revealed that TAZ (WWTR1) is constitutively degraded by the proteasome. Examination of TAZ expression in 40 human cancer cell lines revealed that TAZ protein levels correlate with the invasiveness of breast cancer cell lines, enabling us to demonstrate that TAZ is important for migration, invasion and tumorigenesis of breast cancer cells. Our recent experiments suggest that TAZ, like YAP, is sufficient to transform MCF10A as assessed by anchorage-independent growth in soft agar. As TAZ is also a downstream target inhibited by the Hippo tumor suppressor pathway, S89A mutation rendered TAZ refractory to Hippo inhibition and became more tumorigenic in MCF10A cells. Since TAZ is implicated in various developmental and cellular processes via interacting with different proteins (mostly transcriptional factors), we have investigated the mechanism responsible for TAZ to transform MCF10A cells. Our recent results suggest that endogenous TEAD proteins are important for TAZ to transform MCF10A cells and mutations of TAZ that abrupt its interaction with TEADs abolished its transforming ability. These results suggest that TAZ interacts with TEAD proteins to mediate its transforming ability. Since TEAD4 gene is amplified and its transcript is increased in several cancers, TEAD4 is likely the major partner for TAZ in cellular transformation.

## **Elucidation of the effect of *FAT* knock-down on Hippo pathway in U87MG**

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*FAT* is a known tumor suppressor gene in *Drosophila* and acts as an upstream regulator of hippo pathway. The importance of this pathway is emphasized by its evolutionary conservation and by increasing evidence that its deregulation occurs in various human tumors. Alteration of *FAT* has also been reported in various tumors like oral cancer, breast cancer etc. and a study by our group showed alteration of *FAT* expression in primary human glial tumors. In this study we attempt to characterize the effects of *FAT* knockdown by siRNA system on hippo pathway in human glioma cell line U87MG. The expression of *FAT* mRNA and other mammalian hippo pathway homologs like Mst (hippo), Lats (warts) and YAP (yorkie) was analyzed by real-time PCR. We observed that after 72 hrs of transient transfection, *FAT* siRNA treated cells showed considerable morphological changes as compared to scrambled siRNA treated cells. Expression analysis of *FAT* after 72 hrs of *FAT* siRNA treatment showed about 90% decrease as compared to the scrambled siRNA treated cells. Among the hippo pathway molecules analyzed, Mst showed a 65% decrease in expression while Lats and YAP expression was unaltered at transcriptional level in siRNA treated cells as compared to scrambled siRNA treated cells. Considering the fact that regulation of hippo pathway primarily occurs at post-translational level and depends upon phosphorylation status of the molecules, we are in process of analyzing the expression of these molecules at protein level and their phosphorylation status using phospho-specific antibodies which will provide a better understanding of the pathway.

## **YAP Dysregulation by $\Delta$ Np63-mediated Gene Repression or Phosphorylation Promotes Proliferation, Survival and Migration in Head and Neck Cancer\***

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Yes-associated protein (YAP) has been paradoxically implicated as an oncogene or as a critical nuclear co-factor for p73-mediated apoptosis. We previously observed differential expression and cytoplasmic-nuclear distribution of YAP in different subsets of head and neck squamous cell carcinomas (HNSCC), but the mechanisms underlying these differences in expression and distribution, and the function of YAP remained undetermined. In this study, we found that a subset with elevated, predominately cytoplasmic YAP expression, exhibits low expression of p53 family members TP53,  $\Delta$ Np63 and p73, while another subset exhibits weaker, nuclear YAP expression, together with overexpression of mutant (mt) TP53,  $\Delta$ Np63 and p73. Repression of YAP expression was related to overexpression of  $\Delta$ Np63, as  $\Delta$ Np63 siRNA significantly increased YAP mRNA and protein expression, and  $\Delta$ Np63 bound to the YAP promoter in chromatin immunoprecipitation (ChIP) assay. Cytoplasmic sequestration of YAP was related to phosphorylation of AKT-serine-473 and YAP-serine-127. Increasing nuclear YAP by transfection of a YAP-serine-127-alanine AKT phosphoacceptor-site mutant or YAP transcription by  $\Delta$ Np63 siRNA significantly increased cell death, while knocking down YAP enhanced cell proliferation, survival, migration, and decreased chemosensitivity to cisplatin. Thus, YAP functions as a tumor suppressor in HNSCC, and is predominantly dysregulated by  $\Delta$ Np63-mediated repression of expression or cytoplasmic sequestration by protein phosphorylation in different subsets. These findings suggest that differences in YAP function may be related to transcriptional or post-translational mechanisms, and that  $\Delta$ Np63 and/or AKT may represent important targets for enhancing YAP-mediated apoptosis and chemosensitivity.

## **Dual role of RASSF6 in mammalian Hippo signaling**

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Fly RASSF (dRASSF) blocks the binding of Salvador to Hippo to antagonize pro-apoptotic Hippo signaling. dRASSF loss of function enhances cell growth in Hippo mutant lacking the SARAH domain and recovers cell growth in Ras1 mutant. Here dRASSF paradoxically functions as a tumor suppressor. More confusingly dRASSF loss of function does not enhance cell growth in the kinase-dead Hippo mutant. In contrast to dRASSF, RASSF1A activates Hippo signaling to induce apoptosis. RASSF6, one of mammalian RASSFs, and human Salvador (WW45) bind to distinct sequences of MST2, a homologue of Hippo, to form a tripartite complex. The complex is disrupted when MST2 is activated. RASSF6 and MST2 inhibit each other through the interaction. MST2 prevents RASSF6-induced apoptosis. WW45 cancels the inhibitory effect of MST2. Conversely RASSF6 inhibits MST2 and blocks the activation of Nuclear Dbf2-related (NDR) kinases. RASSF2, 3 and 4 also inhibit MST2, whereas RASSF1A activates it. The chimera of N-terminal RASSF1A and C-terminal RASSF6 activates MST2, indicating that RASSF1A has a distinct property from other RASSFs. RASSF6-induced apoptosis is independent of NDR kinases and partially depends on MOAP1. MST2 attenuates the interaction of RASSF6 with MOAP1, while the addition of WW45 recovers it. Our findings support the model that RASSF6, a prototypic RASSF, plays a dual role as a negative regulator for Hippo signaling and a mediator of apoptosis, which is triggered upon the activation of Hippo signaling. This model may explain why dRASSF exhibits tumor-suppressive property with Hippo lacking the SARAH domain, but not with the kinase-negative Hippo.

## **The spatiotemporal regulation of components of the Hippo pathway during epithelial differentiation in mammals**

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The Hippo pathway has been implicated in the restriction of cell proliferation and in apoptosis for proper development in *Drosophila*, yet the role and molecular mechanisms of this pathway are not fully understood in mammals. We generated WW45(a Salvador homolog)-null mice and revealed the crucial role of Hippo signaling pathway in epithelial development. Many mutant organs displayed expansion of epithelial cells with impaired terminal differentiation. This expansion of progenitor cells in the mutant epithelial tissues was due to impaired proliferation arrest of epithelial cells rather than intrinsic acceleration of proliferation during differentiation. Importantly, the Hippo signaling pathway is activated in epithelial primary cells during differentiation. At the molecular level, WW45 mediates MST1(a Hippo homolog) translocation to the nucleus and activation of LATS1/2 (a Warts homolog), which then phosphorylate serine 127 of YAP (a Yorkie homolog) upon differentiation. This phosphorylation of YAP and subsequent its translocation from the nucleus into the cytoplasm occurs during differentiation in an Hippo signaling dependent manner in vitro as well as in vivo. In addition, nonphosphorylated YAP S127A mutant was mainly localized to the nucleus and its overexpression failed to induce proliferation arrest and differentiation in primary keratinocytes even in a differentiated condition. We will discuss the spatiotemporal regulation of components of the Hippo signaling during differentiation and the role of this pathway in the coupling of proliferation stop with terminal differentiation in mammals.

## **Mst2 regulation is a point of crosstalk between Akt, Raf1 and Rassf1A pathways.**

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The mammalian sterile20-like kinases 1 and 2 (Mst1 and Mst2) have been described as involved in stress-induced apoptosis signalling. Our previous studies demonstrated that Mst2 was inhibited by Raf-1 binding, and that its activation required a switch from Raf-1 to the tumour suppressor Rassf1A. In this study, we investigated the role of the pro-survival Akt/PKB pathway in the regulation of Mst2 function. We showed that Mst2 was phosphorylated by Akt, resulting in the formation of Raf-1/Mst2 complex and in inhibition of Mst2-triggered apoptosis. We identified two Akt phosphorylation sites (Thr117 and Thr384) in Mst2 and generated non-phosphorylated mutants (T117A, T384A and T117/384AA). Single mutants displayed less Akt-induced phosphorylation and double mutant showed no phosphorylation. Moreover, Mst2 mutants were less capable of interacting with Raf-1 and scaffolding protein 14-3-3, and were more recruited by Rassf1A, with enhanced effects observed more with the double mutant. Finally, Mst2 mutants were more active, able to stimulate downstream stress signalling pathways (JNK and p38 MAPK) and showed a stronger pro-apoptotic activity. Taken together, our data showed that Akt/PKB pathway plays a key role in the regulation of Mst2 pro-apoptotic function.

## **YAP is involved in mesothelioma cell development and negatively regulated by Merlin**

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Malignant pleural mesothelioma (MPM) is an aggressive neoplasm associated with asbestos exposure. Despite recent advancements in chemotherapy, the prognosis of patients with MPM remains unfavorable. To determine the key genes responsible for the development of MPM, we performed BAC-array-comparative genome hybridization analysis and found two MPM cases with chromosome 11q22 amplification. The *YAP* gene encoding a transcriptional co-activator was localized in this region and overexpressed in both cases, suggesting it as a candidate oncogene. We analyzed the involvement of YAP in MPM proliferation, as well as its functional and physical interaction with Merlin encoded by the *neurofibromatosis type 2 (NF2)* gene, which is genetically inactivated in 40-50% of MPM cases. YAP-RNAi suppressed growth of a mesothelioma cell line NCI-H290 with *NF2* deletion, through cell-cycle arrest and apoptosis induction, while YAP transfection promoted the growth of MeT-5A, an immortalized mesothelial cell line. We also found that the introduction of *NF2* into NCI-H290 induced phosphorylation at Ser127 of YAP, which was accompanied by reduction of nuclear localization of YAP. However, nuclear localization of a YAP S127A-mutant was not affected by *NF2* induction. These results suggest that YAP is involved in mesothelial cell growth, and that the transcriptional co-activator activity of YAP is functionally inhibited by Merlin. Future studies of transcriptional targets of YAP in MPMs may shed light on the molecular mechanisms of MPM development and lead to new therapeutic strategies.

## **Yap1 inhibits myotube formation in C2C12 cells**

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Yap is a transcriptional co-activator that has been implicated in the control of organ size in *Drosophila melanogaster* and mammals. The aim of our study was to investigate the role of Yap in skeletal muscle. We first screened a mouse tissue library and found ubiquitous expression of the mammalian Hippo pathway members in all organs and several types of skeletal muscle. We then used the C2C12 skeletal myogenic cell line to investigate the role of Yap1 during myogenesis. We observed a significant increase in Yap Ser127 phosphorylation during myogenesis from sub-confluent myoblasts to fully differentiated myotubes. In line with this, Yap was located mainly in the nucleus of myoblasts and increasingly translocated to the cytoplasm during myogenesis, suggesting Yap inactivation during myogenesis. In order to study whether such Yap inactivation has a functional significance, we transfected C2C12 myoblasts with either wild type hYAP1 or constitutively active hYAP1 S127A and induced myogenic differentiation. Cells transfected with constitutively active hYAP1 S127A showed much reduced myotube formation when compared with cells transfected with empty vector or wild type hYAP1. Our findings suggest that Yap becomes inactivated during C2C12 myogenesis and that this inactivation is necessary for myotube formation.

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