Yes-Associated Protein (YAP) Promotes the Nuclear Import of p73

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ABSTRACT

p73 has been identified as a structural and functional homolog of the tumor suppressor p53. However, mechanisms that regulate the localization of p73 have not been fully clarified. The Yes-associated protein (YAP) is a transcriptional coactivator. As a transcriptional coactivator, YAP needs to bind transcription factors to stimulate gene expression. p73 is a reported YAP target transcription factors and YAP has been shown to positively regulate p73 in promoting apoptosis. Previous studies show that p73 interacts with YAP through its PPPY motif, and increases p73 transactivation of apoptotic genes. In this study, we focused on YAP’s regulation of the localization of p73. After transient transfection into Rat pheochromocytoma (PC12) cells and Human embryonic kidney 293T cells with GFP-YAP and/or YFP-p73, and incubated for 24 hours expression. p73 was fused to YFP to allow the examination of its subcellular localization. When expressed alone, YFP-p73 was distributed throughout the cell. When coexpressed with YAP, nuclear accumulation of YFP-p73 became evident. We quantitated the effect of YAP on the redistribution of YFP-p73 by counting cells with nuclear-only YFP signal. We found that YAP can influence the subcellular distribution of p73. Altogether, coexpression with YAP affected the subcellular distribution of the p73 protein. Our studies attribute a central role to YAP in regulating p73 accumulation and YAP, at least in part, might promote the nuclear import of p73.

Keywords: YAP, p73, nuclear import

1 INTRODUCTION

The p73 protein is a transcription factor related to the tumor suppressor p53 and shares the proapoptotic function of p53 [1]. Like p53, p73 is activated by DNA damage and can stimulate p53-independent apoptosis [2]. Gerry Melino et al. [3] have proved that p73 elicits apoptosis via the mitochondrial pathway using PUMA and Bax as mediators. Most studies report that p73 predominately mediate transcription of Bax in response to DNA damaging agents and the resulting cell death [4,5,6,7,8]. However, mechanisms that regulate the localization of p73 have not been fully clarified.

Studies by Yagi et al. [9] have proved that the Yes-associated protein (YAP) is a transcriptional coactivator. As a transcriptional coactivator, YAP needs to bind transcription factors to stimulate gene expression. Reported YAP target

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transcription factors include TEAD, p73, Runx2, and the ErbB4 cytoplasmic domain[9,10,11,12]. Previous other studies show that YAP interacts with the p53 family member p73, resulting in an enhancement of p73’s transcriptional activity[10], and that YAP promotes p73-mediated transcription of proapoptotic genes in response to DNA damaging agents and the resulting cell death [4,5,13,14,15]. Thus, YAP recruitment is a critical event in turning on the transcriptional activity of p73 by favoring the formation of transcriptionally active competent complexes that play a pivotal role in eliciting apoptosis in response to anticancer treatment [4,5,10,13,14,15].

In this study, we focused on YAP’s regulation of the localization of p73. After transient transfection into Rat pheochromocytoma (PC12) cells and 293T cells with GFP-YAP and/or YFP-p73, and incubated for 24 hours expression. p73 was fused to YFP to allow the examination of its subcellular localization. When expressed alone and coexpressed with YAP, we quantitated the effect of YAP on the redistribution of YFP-p73 by counting cells with nuclear-only YFP signal.

2 Materials and Methods

2.1 Cell Culture

The Rat pheochromocytoma (PC12) were cultured in a humidified (5% CO₂, 37°C) incubator in Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies, Inc.), supplemented with 15% fetal bovine serum, 5% horse serum, penicillin (100 U/ml), and streptomycin (100 µg/ml). Human embryonic kidney 293T cells were cultured under the same condition as PC12 cells but not supplemented with horse serum. The medium was refreshed every other day, and the cells were plated at appropriate densities according to each experimental protocol. Before transfection, the cells were cultured in a custom-built dish. When the cells came to 70-80% confluence, plasmids DNA of GFP-YAP and YFP-p73 were transfected with Lipofectamine TM 2000 reagent into the PC12 and 293T cells.

2.2 Chemicals and Plasmids

We used Lipofectin™ 2000 reagent (Invitrogen, USA) to transfect plasmids GFP-YAP and YFP-p73 into PC12/293T cells. The pGFP-YAP was a gift from Prof. Subham Basu, Cell Survival Signalling Laboratory Centre for Molecular Oncology and Imaging Barts and The London School of Medicine and Dentistry, John Vane Science Centre, London and Prof. Julian Downward, Signal Transduction Laboratory, Cancer Research UK London Research Institute, London UK (Subham Basu et al., 2003). The pYFP-p73 was a gift from Prof. Hedeki Shimodaira, Department of Clinical Oncology Institute of Development, Aging and Cancer Tohoku University, Japan (Hedeki Shimodaira et al., 2002 and Ivana Marinovic-Terzic et al., 2008).

2.3 Subcellular Distribution of Yellow Fluorescent Protein (YFP)-Fusion Proteins.

The 293T cells were transfected with pGFP-YAP and/or pYFP-p73 by using Lipofectamine TM 2000. To quantitate the nuclear-only YFP signal, cells were counted and the fluorescence images were collected via a Zeiss C-Apochromat objective (40x, NA=1.3). Cells in 10 randomly chosen fields were scored per experiment.

3 RESULTS

3.1 p73 locates to cytosol and nucleus under physiological conditions
To know the subcellular location of p73, we transfected plasmid YFP-p73 into cells and analyzed the subcellular distribution of fluorescently labeled p73 by confocal microscopy. As is shown in Fig. 1, we found that p73 locates to both cytosol and nucleus under physiological conditions.

![Subcellular localization of YFP-p73 in PC12 and 293T cells.](image)

Figure 1. Subcellular localization of YFP-p73 in PC12 and 293T cells. Cells expressing YFP-p73 were imaged by confocal microscopy. The following specific settings were used for light excitation and emissions: Ar+ laser, Ex. 514 nm, Em. BP LP530 nm.

### 3.2 YAP predominantly locates to cytosol under physiological conditions

Next, we study the subcellular location of YAP, we transfected plasmid GFP-p73 into cells and analyzed the subcellular distribution of fluorescently labeled YAP by confocal microscopy. As is shown in Fig. 2, we found that YAP predominately maintained in cytosol.

![Subcellular localization of GFP-YAP in PC12 and 293T cells.](image)

Figure 2. Subcellular localization of GFP-YAP in PC12 and 293T cells. Cells expressing GFP-YAP were imaged by confocal microscopy. The following specific settings were used for light excitation and emissions: Ar+ laser, Ex. 488 nm, Em. BP BP500-550 nm.

### 3.3 YAP promote the nuclear import of p73

Furthermore, we study on YAP’s regulation of the localization of p73. After transient transfection into PC12 cells and 293T cells with GFP-YAP and/or YFP-p73, and incubated for 24 hours expression. p73 was fused to YFP to allow the examination of its subcellular localization. When expressed alone, we found that YFP-p73 was distributed throughout the cell. When coexpressed with YAP, we found that nuclear accumulation of YFP-p73 became evident. We quantitated the
effect of YAP on the redistribution of YFP-p73 by counting cells with nuclear-only YFP signal. As is show in Figure 3. we found that YAP can influence the subcellular distribution of p73, and at least in part, might promote the nuclear import of p73.

A.

B.
Figure 3. YAP promotes the nuclear import of p73.

A. After transient transfection into PC12 cells and 293T cells with GFP-YAP and/or YFP-p73, Cells were imaged by confocal microscopy. The following specific settings were used for light excitation and emissions: Ar⁺ laser, Ex: GFP-YAP, 458 nm; YFP-p73, 514 nm; Em: GFP-YAP, BP500-530; YFP-p73, LP530.

B. Subcellular Distribution of Yellow Fluorescent Protein (YFP)-Fusion Proteins. To quantitate the nuclear-only YFP signal, cells were counted and the fluorescence images were collected via a Zeiss C-Apochromat objective (40x, NA=1.3). Cells in 10 randomly chosen fields were scored per experiment.

4 DISCUSSION

p73 has been identified as a structural and functional homolog of the tumor suppressor p53. However, mechanisms that regulate the localization of p73 have not been fully clarified. Previous studies show that p73 is a reported YAP target transcription factors, and YAP interacts with p73, resulting in an enhancement of p73’s transcriptional activity[4,5,10,13,14,15]. Thus, we speculated that YAP could regulate the localization of p73. After transient transfection into Rat pheochromocytoma (PC12) cells and 293T cells with GFP-YAP and/or YFP-p73, and incubated for 24 hours expression. p73 was fused to YFP to allow the examination of its subcellular localization. When expressed alone, we found that YFP-p73 was distributed throughout the cell (Figure 1). When coexpressed with YAP, nuclear accumulation of YFP-p73 became evident (Figure 3). We quantitated the effect of YAP on the redistribution of YFP-p73 by counting cells with nuclear-only YFP signal. We found that YAP can influence the subcellular distribution of p73. Altogether, coexpression with YAP affected the subcellular distribution of the p73 protein. Our studies attribute a central role to YAP in regulating p73 accumulation and YAP, at least in part, might promote the nuclear import of p73.

Some studies have reported that laser irradiation diverse signaling pathways and ultimately affects the cell physiological processes [16]. Consistent with Ca²⁺ increase [17], Gao et al. shown that PKC kinases are activated in human lung adenocarcinoma (ASTC-a-1) cells [18] and rat pheochromocytoma (PC12) cells [19] in response to Low-power laser irradiation (LPLI). It is also reported that LPLI triggers a significant activation of ROS/Src pathway [20] and promotes formation of circular dorsal ruffles via PI3K/Ras pathway [21]. Meanwhile, increase of the level of Akt phosphorylation is observed in African green monkey SV40-transformed kidney fibroblast cells (COS-7) when stimulated by LPLI (<50J/cm²) [22-23]. Subham Basu et al.[4] proved that Akt promotes YAP localization to the cytoplasm, resulting in loss from the nucleus where it functions as a coactivator of transcription factors including p73. Thus, we speculated that YAP maintained in cytoplasm by LPLI via PI3K/Akt pathway, and needs further investigation. This extends the range of mechanisms whereby Akt can promote cellular survival in the face of apoptotic stimuli-mediated by YAP.

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REFERENCES

[1] Ozaki T, Nakagawara A., p73, a sophisticated p53 family member in the cancer world. Cancer Sci, 96:729–737(2005).

[2] Leverero M, V. De Laurenzi, A. Costanzo1, S. Sabatini, J. Gong, J. Y. J. Wang and G. Melino, The p53/p63/p73 family of transcription factors: Overlapping and distinct functions. J Cell Sci 113:1661–1670(2000).

[3] Gerry Melino, Francesca Bernassola, Marco Ranalli, Karen Yee, Wei Xing Zong, Marco Corazzari, Richard A. Knight, Doug R. Green, Craig Thompson, and Karen H. Vousden,. p73 Induces Apoptosis via PUMA Transactivation and Bax Mitochondrial Translocation. J Biol Chem, 279(9):8076-8083(2004).

[4] Subham Basu, Nicholas F. Totty, Meredith S. Irwin, Marius Sudol, and Julian Downward, Akt Phosphorylates the Yes-Associated Protein, YAP, to Induce Interaction with 14-3-3 and Attenuation of p73-Mediated Apoptosis. Molecular Cell, 11: 11–23(2003).

[5] Sabrina Strano, Olimpia Monti, Natalia Pedicioni, Alessia Baccarini, Giulia Fontemaggi, Eleonora Lapi, Fiamma Mantovani, Alexander Damalas, Gennaro Citro, Ada Sacchi, Giannino Del Sal, Massimo Levrero, and Giovanni Blandino, The Transcriptional Coactivator Yes-Associated Protein Drives p73 Gene-Target Specificity in Response to DNA Damage. Molecular Cell, 18: 447–459(2005).

[6] Claudie Hooper, Mahvash Tavassoli, J. Paul Chapple, Dafe Uwanogho, Richard Goodyear,Gerry Melino, Simon Lovestone and Richard Killick, TAp73 isoforms antagonize Notch signalling in SH-SY5Y neuroblastomas and in primary neurons. Journal of Neurochemistry, 99: 989–999(2006).

[7] Chee-Onn Leong,1 Nick Vidnovic, Maurice Phillip DeYoung, Dennis Sgroi and Leif W. Ellisen, The p63/p73 network mediates chemosensitivity to cisplatin in a biologically defined subset of primary breast cancers. The Journal of Clinical Investigation, 117:1370–1380(2007).

[8] Dan Levy, Yaarit Adamovich, Nina Reuven, and Yosef Shaul,. Yap1 Phosphorylation by c-Abl Is a Critical Step in Selective Activation of Proapoptotic Genes in Response to DNA Damage. Molecular Cell ,29: 350–361(2008).

[9] Ryoei Yagi, Lin-Feng Chen, Katsuya Shigesada1, Yota Murakami and Yoshiaki Ito., A WW domain-containing Yes-associated protein (YAP) is a novel transcriptional co-activator. The EMBO Journal, 18(9): 2551–2562(1999).

[10] Sabrina Strano, Eliana Munarriz, Mario Rossi, Luisa Castagnolii, Yosef Shaul, Ada Sacchi, Moshe Oren, Marius Sudol, Gianni Cesareni, and Giovanni Blandino, 2001. Physical Interaction with Yes-associated Protein Enhances p73 Transcriptional Activity. J. Biol. Chem., 276(18): 15164–15173.

[11] Alex Vassilev, Kotaro J. Kaneko, Hongjun Shu, Yimingming Zhao, and Melvin L. DePamphilis,. TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm. Genes & Development , 15:1229–1241(2001).

[12] Akihiko Komuro, Makoto Nagai, Nicholas E. Navin, and Marius Sudol, WW Domain-containing Protein YAP Associates with ErbB-4 and Acts as a Co-transcriptional Activator for the Carboxyl-terminal Fragment of ErbB-4 That Translocates to the Nucleus. J. Biol. Chem., 278(35): 33334–33341(2003).

[13] D Levy, Y Adamovich, N Reuven and Y Shaul, The Yes-associated protein 1 stabilizes p73 by preventing Itch-mediated ubiquitination of p73. Cell Death and Differentiation,14: 743–751(2007).

[14] David Matallanas, David Romano, Karen Yee, Katrin Meissl, Lucia Kucerova, Daniela Piazzolla,Manuela Baccarini, J. Keith Vass, Walter Kolch, and Eric O’Neill1, RASSF1A Elicits Apoptosis through an MST2 Pathway Directing Proapoptotic Transcription by the p73 Tumor Suppressor Protein. Molecular Cell, 27: 962–975(2007).
[15] Eleonora Lapi, Silvia Di Agostino, Sara Donzelli, Hilah Gal, Eytan Domany, Gideon Rechavi, Pier Paolo Pandolfi, David Givol Sabrina Strano, Xin Lu, and Giovanni Blandino, PML, YAP, and p73 Are Components of a Proapoptotic Autoregulatory Feedback Loop. *Molecular Cell*, 32: 803–814 (2008).

[16] Gao, X., Xing, D., Molecular mechanisms of cell proliferation induced by low power laser irradiation. *J Biomed Sci*, 12, 16-24 (2009).

[17] Lavi, R., Shainberg, A., Friedmann, H., Shneyvays, V., Rickover, O., Eichler, M., Kaplan, D., and Lubart, R., Low energy visible light induces reactive oxygen species generation and stimulates an increase of intracellular calcium concentration in cardiac cells. *J Biol Chem*, 278, 40917–40922 (2003).

[18] Gao, X., Chen, T., and Xing, D., Wang, F., Pei, Y., and Wei, X., Single cell analysis of PKC activation during proliferation and apoptosis induced by laser irradiation. *J Cell Physiol*, 206, 441–448 (2006).

[19] Zhang, L., Xing, D., Zhu, D., and Chen, Q., Low-Power Laser Irradiation Inhibiting Aβ25-35-induced PC12 Cell Apoptosis via PKC Activation. *Cell Physiol Biochem* 22, 215–222 (2008).

[20] Zhang, J., Xing, D., Gao, X., Low-Power Laser Irradiation activates Src tyrosine kinase through reactive oxygen species-mediated signaling pathway. *J Cell Physiol*, 217(2), 518-28 (2008).

[21] XUEJUAN GAO, DA XING,* LEI LIU, AND YONGHONG TANG, H-Ras and PI3K Are Required for the Formation of Circular Dorsal Ruffles Induced by Low-Power Laser Irradiation. *J Cell Physiol*, 219, 535-543 (2009).

[22] Zhang, LL, Xing, D., Gao, X., and Wu, S., Low-Power Laser Irradiation Promotes Cell Proliferation by Activating PI3K/Akt Pathway. *J Cell Physiol*, 219, 553-562 (2009).

[23] Zhang, LL., Zhang YJ., X., and Xing, D., LPLI Inhibits Apoptosis Upstream of Bax Translocation via a GSK-3β-Inactivation Mechanism. *J Cell Physiol*, 224, 218-228 (2010).