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☐ REVIEW ARTICLE ☐

Are Cyclophilins Foes or Friends?

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Cyclophilins (Cyps) are intracellular proteins originally discovered as cellular binding proteins for immunosuppressive drug, CsA. Recently, many studies report important roles of Cyps in cancer cell biology. Cyclophilin A (CypA) is overexpressed in several types of cancer cell. It has been reported that CypA plays a role in cancer cell proliferation, or anti-apoptosis. Overexpressed CypA protects prostate cancer cells from hypoxia- or cisplatin-induced apoptosis. Also CypA has been known to regulate ERK1/2, Jak2, and Bcl2 signaling. Cyclophilin B (CypB) seems to have much more influence on breast cancer. It has been known that CypB can promote cancer cell proliferation, and its motility. More importantly, CypB antigenic epitopes have been used against lung cancer in clinical trail phase I. CypD and Cyp40 also have been studied in several cancer cells. CypD reduces mitochondria mediated apoptosis and Cyp40 positively modulates androgen-dependent prostate cancer cell growth. This review will mainly discuss biological functions of CypA and CypB in oxidative stress and human cancers. Also, we will briefly discuss the recent findings on the importance of other Cyps in human cancers. This study will eventually lead us to find valuable strategies for cancer treatment and prevention. **(*Cancer Prev Res* 15, 101-105, 2010)**

Key Words: Oxidative stress, Cancer, Cyclophilns

BACKGROUND OF CYCLOPHILINS AND OXIDATIVE STRESS

Cyclophilins (Cyps) (16 members in humans), are members of the immunophilin family of proteins that show peptidyl-prolyl *cis*-trans isomerase (PPIase) activity. They are abundant, ubiquitously expressed proteinsoriginally discovered as an intracellular ligand of the immunosuppressive drug cyclosporin A (CsA). Cyps have been found in all prokaryotes, eukaryotes, and plants, and structurally conserved throughout the evolution, implying their importance in cellular functions. Their Peptidyl-prolyl isomerases (PPIase) activity catalyzes the *cis*-trans interconversion of imide bonds of proline residues.[1] The PPIase-containing proteins are collectively known as immunophilins, and also include FK506-binding proteins and parvulins as well as Cyps.[2]

Oxygen free radical, more generally known as reactive oxygen species (ROS) are well recognized for playing a dual role as both deleterious and beneficial species. Target of the free radicals include all kinds of molecules in the body. Among them lipid, nucleic acids and proteins are the major targets.[3] ROS within cells act as secondary messengers in intracellular signalling cascades, which induce and maintain the oncogenic phenotype of cancer cells. However, ROS can also induce cellular senescence and apoptosis and can, therefore, function as anti-tumourigenic species. ROS can directly produce single- or double-stranded DNA breaks, purine-, pyrimidine-, or deoxyribose- modifications, and DNA cross-links. Persistent DNA damage can result in cell cycle arrest, abnormal gene

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expression, replication errors, and genomic instability, all of which are seen in cancer cells. Important to cancer cells, the unregulated or prolonged production of cellular oxidants has been linked to mutation induced by oxidant-induced DNA damage, as well as modification of gene expression.[4~6] Evidence that cyclophilins function as an antioxidant has been recently accumulated. Choi *et al*. showed that overexpressed CypA removed ROS generated by cyclosporine A.[7] Moreover, CypA has been reported to be secreted in response to ROS in vascular smooth muscle cells. It can be hypothesized that in higher amount of Cyclophilins might be required to accelerate folding and maturation of newly synthesized protein with ROS protective functions. Therefore, maybe cyclophilins have protective functions against ROS, for example, by eliminating radicals.

RELATIONSHIP OF CYCLOPHILINS AND CANCER

Many cyclophilns-related study have been focused in several fields of research including HIV, HCV, cardiovascular, and inflammatory diseases. However, evidences are accumulated that cyclophlins have strong relationship with cancer (Table 1).

Cyclophlin A (CypA) is associated with many human cancers.[8] Using SAGE (Serial analysis of gene expression), they found that CypA mRNA expression profile is different in some tissue types. In Brain,[9~12] Breast,[13~15] colon,[16] kidney,[10] stomach,[10~17] skin,[18] and many other cancer tissues, CypA mRNA was overexpressed. They suggested that CypA is very important molecule in cancer pathology.

According to recent studies, CypA are discussed as a target factor of tumorigenesis. In prostate cancer, CypA up-regulation is mediated by hypoxia-inducible-factor 1α (HIF-1α). And overexpresssion of CypA prevented hypoxia- and cisplatin-induced apoptosis, and this was associated with the reduction of oxidative stress and mitochondria damage.[19] When CypA expression level was suppressed, the proliferation ratio was decreased 47.8% of the control value inendometrial carcinoma. Furthermore, clonogenic formation assay demonstrated that knock-down CypA group showed 68.8% inhibition ratio compared with control groups. And *in vitro*, similarly to *in vivo* results, PCNA (Proliferating cell nuclear antigen) positive nuclei in tumors were over 56% smaller than controls.[20]

Although the role of CypA in apoptosis is not clear at this point, several reports show that CypA is released from cardiac myocytes in response to hypoxia/reoxygenation and may protect cardiac myocytes from oxidative stress-induced apoptosis,[21] and that CypA also protects other cells from oxidative stress-induced apoptosis.[22] These reports suggest that CypA might be important for tumorigenesis in solid tumors. Interestingly, overexpression of CypA prevented hypoxia- and cisplatin-induced apoptosis, and this obsevation was associated with the suppression of reactive oxygen species generation and depolarization of mitochondrial membrane potential, whereas small interfering RNA-based CypA knockdown aggravated these factors. These results suggest that CypA is important in tumorigenesis, especially in tumor apoptosis.

From the molecular biology viewpoint, CypA is known to stimulate ERK1/2 signal in small lung cancer. The ERK 1/2 pathway is well known to stimulate cell growth by growth factors and plays an important role in cancer cell growth and proliferation.[23] And also CypA is known to regulate Janus-Activated Kinase 2, which results in PRLr/growth hormone receptor phosphorylation and the activation of several signaling including STAT5, Ras/MAP kinase, and Nek3/Var2/Rac1 pathway. In this study, CypA contributes to the activation of Jak2 and other PRL-r associated signaling pathways, through binding of ligand to the PRLr as a receptor-associated conformational switch. Loss of PRLr-CypA binding resulted in a loss of PRLr/Jak2 mediated signaling and inhibited their growth, motility, and invasion in soft agar colony formation.[24]

In pancreatic cancer, CypA and CD147 expressed significantly higher than normal pancreatic ductal epithelim. CD147 is type I transmembrane glycoprotein, and when CypA binds to CD147, it transmits a signal to downstream cascades. Overexpressed CypA stimulates MAP kinase, IL-5 and IL-17 though CD147. As results, CypA promote cell proliferation and tumorigenesis.[25]

Cyclophilin B (CypB) also has been investigated in cancer pathology. Especially in breast cancer, CypB has been considered as an important factor. Retro-tanslocation of CypB from ER to nucleus makes possible for CypB to bind with Prolactin (PRL). The CypB-PRL complex can stimulate STAT5-mediated gene expression. This study showed that CypB as an important protein for the lactogenic hormones. The important role of the PRL-related signaling in the development and progression of breast cancer has been reported by several studies.[26~29]

Table 1. Cyclophilins functions in cancers

Cyclophilins	Cancer type	Functions of Cyps in cancer
CypA	Pancreatic cancer	- Upregulated as compared with normal pancreatic control tissues - Stimulates cell proliferation through CD147 - Involved in cell proliferation such as neuropilins (NRPs), vascular endothelial growth factor (VEGF), and VEGF receptors (VEGFRs)
	Oral squamous cancer	- HIF-1α expression indicates a good prognosis in early stage - Differential gene expression in neoplastic
	Small lung cancer	- Potential role of CypA in early stage neoplastic transformation - Involved in cell cycle progression and MAPKs pathway activation
	Colorectal cancer	- COX2-independent chemopreventive effect by celecoxib
CypB	T lymphocytes	- Recognized by HLA-A24-restricted and tumor specific cytotoxic T lymphocytes
	Breast cancer	- Served as a transcription inducer
	Lung cancer	- Peptide vaccines against lung cancer in a clinical trial
CypC	Breast cancer	- Marker of metastatic function
Cyp40	Prostate cancer	- Interacts with Bcl2 - Positive regulators of androgen-dependent
	Breast cancer	- Estrogen receptor α/β subtype expression and interacting hsp90
CypD	Cervical carcinoima	- Sirtuin-3-induced inactivation of CypD causes a detachment of hexokinase II from the mitochondria - Need for oxidative phosphorylation
	Ovarian cancer	- Mitochondiral chaperone TRAP1 regulates potential novel target for ovarian cancer therapy

The effect of CypB gene expression and global cellular function in human breast cancer has been studied by proteomic methods. According this report, CypB knockdown regulate cell motility, proliferation, essential cell function, and many cancer biology related-network genes in breast cancer cells. *In vivo*, CypB levels are increased in malignant breast epithelium and significant concentration within the nucleus.[30)]

In 2002, Gohara *et al*. found that two CypB antigenic epitopes (CypB 84～92 and CypB 91～99) are recognized by HLA-A24-restricted and tumor-specific cytotoxic T lymphocyte. These peptide-based vaccines were used against lung cancer in clinical trial.[31)]

Also recently it turned out that CypB are strongly related with Hepatitis C Virus (HCV) and hepato cellular carcinoma (HCC). Progression of the disease was determined on biopsy using the development of the end-stage complications of progressive hepatitis C, namely development of HCC.[32)] It was reported that CypB is a cofactor of HCV replication, and that CypB and viral non-structural proteins are complexes for viral replication.[33)]

Cyclophilin D (CypD) and Cyclophilin 40 (Cyp40) were investigated in several cancers. Overexpressed CypD was known to inhibit proliferation of cancer cells.[34～36)] In glioma and cervix cancer, mitochondria-mediated apoptosis were reduced by CypD suppression. They found that anti-cancer drug (adriamycin)-mediated apoptosis suppression by CypD correlated with amount of mitochondrial bound hexokinase II, which has anti-apoptotic activity.[37)] And CypD interacts with Bcl2, which is well-known as anti-apoptotic factor. In sarcoma osteodenic and leukemia cell, CypD enhances the limiting effect of Bcl2 on the cytochrome C releases from mitochondria which is not mediated via mitochondrial permeability transition (MPT).[38)]

Cyp40 were studied in prostate cancer.[39～42)] Cyp40 is much highly expressed in prostate cancer cell lines compared with primary prostate cells, and Cyp40 associated with AR.[41)] Knockdown of Cyp40 inhibited androgen (AR)-mediated transcription and growth in prostate cancer cell, LNCaP.[42)]

CONCLUSION

ROS (Reactive oxygen species) are known to play dual roles in biological system, since they can be either harmful or beneficial in living systems. At low concentrations of ROS, they are known to play a beneficial function in the induction of a mitogenic response, while at high concentration, they can be important mediators for cellular damage to lipid, protein and

nucleic acids. Therefore, ROS have been proposed to play key roles in the development of age-dependent disease such as cancer. Cyps are originally identified as cellular binding proteins for the immunosuppressive drug cyclosporin A. It was known that Cyps, including CypA, CypB, CypC. CypD and Cyp40, have different locations and functions. Clinically, Cyps have been studied in many diseases, such as Parkinson's diseases, Alzheimer's diseases, and Hepatitis C virus, which have strong correlation with oxidative stress. In fact, one of Cyps' roles in diseases turned out to be related with oxidative stress, such as mitochondria dysfunction, ROS reductions. Furthermore, nowadays, the expression patterns and functions of Cyps are reported to be correlated with tumor biology of several cancers. Even though exact mechanisms of Cyps in oxidative stress-related diseases are to be revealed, understanding the roles of Cyps in oxidative stress and cancers will lead to finding their clinical applications in human disease.

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REFERENCES

1) Gothel SF, Marahiel MA. Peptidyl-prolyl cis-trans isomerases, a superfamily of ubiquitous folding catalysts. *Cell Mol Life Sci* 55, 423-436, 1999.
2) Dolinski K, Scholz C, Muir RS, Rospert S, Schmid FX, Cardenas ME, Heitman J. Functions of FKBP12 and mitochondrial cyclophilin active site residues *in vitro* and *in vivo* in Saccharomyces cerevisiae. *Mol Biol Cell* 8, 2267-2280, 1997.
3) Poli G, Leonarduzzi G, Biasi F, Chiarpotto E. Oxidative stress and cell signalling. *Curr Med Chem* 11, 1163-1182, 2004.
4) von Sonntag C. New aspects in the free-radical chemistry of pyrimidine nucleobases. *Free Radic Res Commun* 2, 217-224, 1987.
5) Dizdaroglu M. Oxidative damage to DNA in mammalian chromatin. *Mutat Res* 275, 331-342, 1992.
6) Demple B, Harrison L. Repair of oxidative damage to DNA: enzymology and biology. *Annu Rev Biochem* 63, 915-948, 1994.
7) Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 408, 239-247, 2000.
8) Obchoei S, Wongkhan S, Wongkham C, Li M, Yao Q, Chen C. Cyclophilin A: potential functions and therapeutic target for human cancer. *Med Sci Monit* 15, RA221-232, 2009.
9) Velculescu VE, Zhang L, Vogelstein B, Kinzler KW. Serial analysis of gene expression. *Science* 270, 484-487, 1995.
10) Lal A, Lash AE, Altschul SF, Velculescu V, Zhang L, McLendon RE, Marra MA, Prange C, Morin PJ, Polyak K, Papadopoulos N, Vogelstein B, Kinzler KW, Strausberg RL, Riggins GJ. A public database for gene expression in human cancers. *Cancer Res* 59, 5403-5407, 1999.
11) Boon K, Eberhart CG, Riggins GJ. Genomic amplification of orthodenticle homologue 2 in medulloblastomas. *Cancer Res* 65, 703-707, 2005.
12) Boon K, Edwards JB, Siu IM, Olschner D, Eberhart CG, Marra MA, Strausberg RL, Riggins GJ. Comparison of medulloblastoma and normal neural transcriptomes identifies a restricted set of activated genes. *Oncogene* 22, 7687-7694, 2003.
13) Porter DA, Krop IE, Nasser S, Sgroi D, Kaelin CM, Marks JR, Riggins G, Polyak K. A SAGE (serial analysis of gene expression) view of breast tumor progression. *Cancer Res* 61, 5697-5702, 2001.
14) Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, Nikolskaya T, Serebryiskaya T, Beroukhim R, Hu M, Halushka MK, Sukumar S, Parker LM, Anderson KS, Harris LN, Garber JE, Richardson AL, Schnitt SJ, Nikolsky Y, Gelman RS, Polyak K. Molecular definition of breast tumor heterogeneity. *Cancer Cell* 11, 259-273, 2007.
15) Seth P, Krop I, Porter D, Polyak K. Novel estrogen and tamoxifen induced genes identified by SAGE (Serial Analysis of Gene Expression). *Oncogene* 21, 836-843, 2002.
16) Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, Vogelstein B, Kinzler KW. Gene expression profiles in normal and cancer cells. *Science* 276, 1268-1272, 1997.
17) Lal A, Peters H, StCroix B, Haroon ZA, Dewhirst MW, Strausberg RL, Kaanders JH, van der Kogel AJ, Riggins GJ. Transcriptional response to hypoxia in human tumors. *J Natl Cancer Inst* 93, 1337-1343, 2001.
18) Weeraratna AT, Becker D, Carr KM, Duray PH, Rosenblatt KP, Yang S, Chen Y, Bittner M, Strausberg RL, Riggins GJ, Wagner U, Kallioniemi OP, Trent JM, Morin PJ, Meltzer PS. Generation and analysis of melanoma SAGE libraries: SAGE advice on the melanoma transcriptome. *Oncogene* 23, 2264-2274, 2004.
19) Choi KJ, Piao YJ, Lim MJ, Kim JH, Ha J, Choe W, Kim SS. Overexpressed cyclophilin A in cancer cells renders resistance to hypoxia- and cisplatin-induced cell death. *Cancer Res* 67, 3654-3662, 2007.
20) Li Z, Zhao X, Bai S, Wang Z, Chen L, Wei Y, Huang C. Proteomics identificationof cyclophilin a as a potential prognostic factor and therapeutic target in endometrial carcinoma. *Mol Cell Proteomics* 7, 1810-1823, 2008.
21) Seko Y, Fujimura T, Taka H, Mineki R, Murayama K, Nagai

R. Hypoxia followed by reoxygenation induces secretion of cyclophilin A from cultured rat cardiac myocytes. *Biochem Biophys Res Commun* 317, 162-168, 2004.
22) Doyle V, Virji S, Crompton M. Evidence that cyclophilin-A protects cells against oxidative stress. *Biochem J* 341(Pt 1), 127-132, 1999.
23) Yang H, Chen J, Yang J, Qiao S, Zhao S, Yu L. Cyclophilin A is upregulated in small cell lung cancer and activates ERK1/2 signal. *Biochem Biophys Res Commun* 361, 763-767, 2007.
24) Zheng J, Koblinski JE, Dutson LV, Feeney YB, Clevenger CV. Prolyl isomerase cyclophilinA regulation of Janus-activated kinase 2 and the progression of human breast cancer. *Cancer Res* 68, 7769-7778, 2008.
25) Li M, Zhai Q, Bharadwaj U, Wang H, Li F, Fisher WE, Chen C, Yao Q. Cyclophilin A is overexpressed in human pancreatic cancer cells andstimulates cell proliferation through CD147. *Cancer* 106, 2284-2294, 2006.
26) Hankinson SE, Willett WC, Michaud DS, Manson JE, Colditz GA, Longcope C, Rosner B, Speizer FE. Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 91, 629-634, 1999.
27) Lapensee E, Ben-Jonathan N. Novel roles of prolactin and estrogens in breast cancer: resistance to chemotherapy. *Endocr Relat Cancer* 17, R91-R107, 2010.
28) LaPensee EW, Schwemberger SJ, LaPensee CR, Bahassi el M, Afton SE, Ben-Jonathan N. Prolactin confers resistance against cisplatin in breast cancer cells by activating glutathione-S-transferase. *Carcinogenesis* 30, 1298-1304, 2009.
29) Clevenger CV, Furth PA, Hankinson SE, Schuler LA. The role of prolactin in mammary carcinoma. *Endocr Rev* 24, 1-27, 2003.
30) Fang F, Flegler AJ, Du P, Lin S, Clevenger CV. Expression of cyclophilin B is associated with malignant progression and regulation of genes implicated in the pathogenesis of breast cancer. *Am J Pathol* 174, 297-308, 2009.
31) Gohara R, Imai N, Rikimaru T, Yamada A, Hida N, Ichiki M, Kawamoto M, Matsunaga K, Ashihara J, Yano S, Tamura M, Ohkouchi S, Yamana H, Oizumi K, Itoh K. Phase 1 clinical study of cyclophilin B peptide vaccine for patients with lung cancer. *J Immunother* 25, 439-444, 2002.
32) Sherman M. Hepatitis C and hepatocellular carcinoma: grist for the mill. *Gastroenterology* 136, 39-42, 2009.
33) Heitman J, Cullen BR. Cyclophilin B escorts the hepatitis C virus RNA polymerase: a viral achilles heel? *Mol Cell* 19, 145-146, 2005.
34) Lin DT, Lechleiter JD. Mitochondrial targeted cyclophilin D protects cells from cell death by peptidyl prolyl isomerization. *J Biol Chem* 277, 31134-31141, 2002.
35) Schubert A, Grimm S. Cyclophilin D, a component of the permeability transition-pore, is an apoptosis repressor. *Cancer Res* 64, 85-93, 2004.
36) Li Y, Johnson N, Capano M, Edwards M, Crompton M. Cyclophilin-D promotes the mitochondrial permeability transition but has opposite effects on apoptosis and necrosis. *Biochem J* 383, 101-109, 2004.
37) Machida K, Ohta Y, Osada H. Suppression of apoptosis by cyclophilin D via stabilization of hexokinase II mitochondrial binding in cancer cells. *J Biol Chem* 281, 14314-14320, 2006.
38) Eliseev RA, Malecki J, Lester T, Zhang Y, Humphrey J, Gunter TE. Cyclophilin D interacts with Bcl2 and exerts an anti-apoptotic effect. *J Biol Chem* 284, 9692-9699, 2009.
39) Mark PJ, Ward BK, Kumar P, Lahooti H, Minchin RF, Ratajczak T. Human cyclophilin 40 is a heat shock protein that exhibits altered intracellular localization following heat shock. *Cell Stress Chaperones* 6, 59-70, 2001.
40) Hursting SD, Shen JC, Sun XY, Wang TT, Phang JM, Perkins SN. Modulation of cyclophilin gene expression by N-4-(hydroxyphenyl)retinamide: association with reactive oxygen species generation and apoptosis. *Mol Carcinog* 33, 16-24, 2002.
41) Periyasamy S, Warrier M, Tillekeratne MP, Shou W, Sanchez ER. The immunophilin ligands cyclosporin A and FK506 suppress prostate cancer cell growth by androgen receptor-dependent and -independent mechanisms. *Endocrinology* 148, 4716-4726, 2007.
42) Periyasamy S, Hinds T Jr, Shemshedini L, Shou W, Sanchez ER. FKBP51 and Cyp40 are positive regulators of androgen-dependent prostate cancer cell growth and the targets of FK506 and cyclosporin A. *Oncogene* 29, 1691-1701, 2009.