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Tumor suppressor p16^{INK4a} in Cancer

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Tumor suppressor p16^{INK4a} in Cancer

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ABSTRACT **: p16^{INK4a} is a tumor suppressor that belongs to the INK4 family of the cyclin D-dependent kinases (cdk) inhibitors. It plays regulatory roles in cell proliferation and in tumorigenesis by interacting with Rb signaling. Abnormally elevated p16^{INK4a} protein expression causes cell cycle arrest (G1/S transition) and loss of cyclin-cdk activity. In many cancers, p16^{INK4a} is altered by mutation, deletion, and promoter methylation. This review summarizes the function of p16 as an important regulator of cancer pathobiology and a promising target for developing cancer therapeutic and chemopreventive agents.**

Key words : **p16^{INK4a}, tumor suppressor, alterations, cancer therapy**

Introduction

세포주기는 G1, S, G2, M phase로 이루어져 있으며, growth factor withdrawal 과 같이 세포 내 환경이 좋아지지 않을 경우에 quiescent state인 G0 phase로 들어가기도 한다 (Fig. 1) (Collins *et al.*, 1997; Sherr, 1995; Sherr and Roberts, 1995). 각각의 phase가 원만히 진행되는 데에는 특이적으로 또는 복합적으로 관여하는 요소들이 있는데,

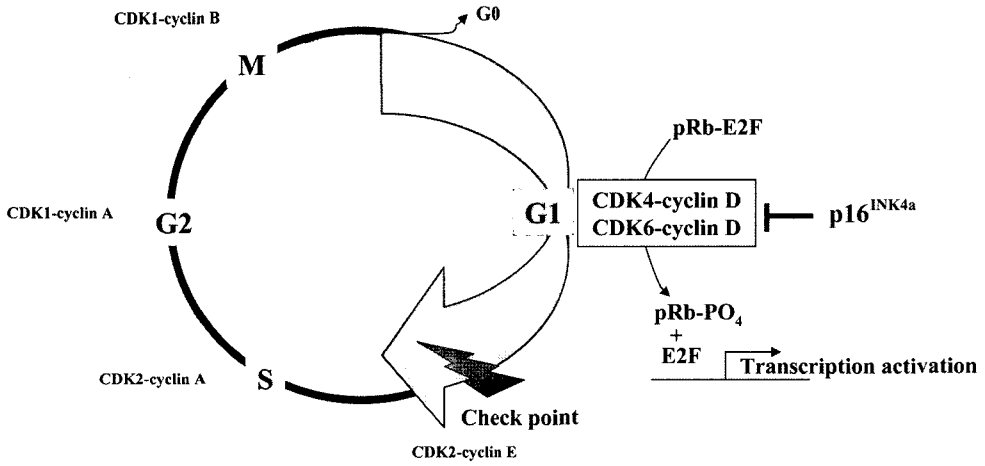

Fig. 1. Mammalian cell cycle and regulation of Rb pathway by p16^{INK4a}.

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Table 1. Human tumor suppressor genes (modified the table of C.J. Sherr, 2004, T. Jacks, 1996)

Gene	Protein function	Inherited syndrome	Sporadic cancers with mutations	References
RB	Transcriptional regulation	Familial retinoblastoma	Retinoblastoma, osteosarcoma, breast, lung and bladder carcinoma	(Weinberg, 1995), (Knudson, 1971),
p53	Transcription factor	Li-Fraumeni syndrome	50% of all tumors	(Greenblatt et al., 1994), (Ko and Prives, 1996)
WT1	Transcriptional regulation; interacts with splicing factors	Wilm's tumor	Nephroblastoma	(Hastie, 1994)
APC	Binds β-catenin	Familial adenomatous polyposis	Colon and stomach carcinoma	(Groden et al., 1991), (Kinzler et al., 1991), (Polakis, 1995)
MSH2 and MLH1	DNA mismatch repair	Hereditary nonpolyposis colorectal cancer (Lynch syndrome)	Endometrial, gastric, ovarian, bladder cancers	(Chung and Rustgi, 2003), (Lengauer et al., 1998), (Loeb et al., 2003)
ATM	DNA damage sensor (protein kinase)	Ataxia telangiectasia	Lymphoreticular malignancies	(Bakkenist and Kastan, 2003), (Shiloh and Kastan, 2001)
NBS1	DNA repair, S phase checkpoint control	Nijmegen breakage syndrome	Lymphoreticular malignancies	(Shiloh and Kastan, 2001)
CHK2	Protein kinase(G1 checkpoint control)	Li-Fraumeni syndrome	?	(Bell et al., 1999)
NF1	Ras-GTPase activating protein activity	Neurofibromatosis type I	Astrocytoma, colon carcinoma, sarcoma, glioma	(Viskochil et al., 1993)
NF2	Membrane cytoskeletal attachment	Neurofibromatosis type II	Schwannoma, meningioma, ependymoma	(Rouleau et al., 1993), (Trofatter et al., 1993)
INK4A/MTS1	Cyclin/cdk inhibitor	Familiar melanoma	Many	(Sherr and Roberts, 1995),
ARF	Mdm2 antagonist (p53 activation)	Melanoma	Many	(Schagdarsurengin et al., 2003), (Aslanian et al., 2004), (Brown et al., 2004)
DCC	N-CAM homology	?	Colon carcinoma	(Cho and Fearon, 1995), (Fearon et al., 1990)
VHL	Interacts with elongation factors, E3 ligase recognition factoe for HIF-1a	von Hippel-Lindau syndrome	Renal cell carcinoma, pheochromocytoma	(Duan et al., 1995), (Kibel et al., 1995), (Latif et al., 1993)
BRCA1	DNA repair (Zinc finger)	Familial breast/ovarian cancer	?	(Miki et al., 1994)
BRCA2	DNA repair	Familial breast cancer	?	(Tavtigian et al., 1996), (Wooster et al., 1995)
TSC1,2	Rap1-GTPase activating protein activity	Tuberous sclerosis	Renal cell carcinoma(rare), angiofibromas	(1993), (Sampson and Harris, 1994)
FHIT	Nucleoside metabolism	?	Esophaogeal, stomach, colon and lung carcinoma	(Ohta et al., 1996), (Sozzi et al., 1996)
DPC4/SMAD4	Signaling molecule in TGF-β pathway	Juvenile polyposis (hamartomas)	Pancreatic and colon cancer	(Hahn et al., 1996b)
PTC	Transmembrane protein	Basal cell nevus syndrome	Basal cell carcinoma	(Hahn et al., 1996a), (Johnson et al., 1996)
SNF5/INI1	Chromatin-remodeling SWI/SNF multiprotein complexes	?	Malignant rhabdoid tumours(MRT)	(Misawa et al., 2004)
PTEN	Lipid phosphatase	Cowden syndrome	Glioblastoma, endometrial, thyroid and prostate cancer	(Maehama and Dixon, 1999), (Sulis and Parsons, 2003)
RASSF1A	Ras effector protein homology	?	Lung, breast, pancreas, kidney, liver, cervix, nasopharyngeal, prostate, thyroid cancers	(Agathanggelou et al., 2001), (Liu et al., 2002), (Schagdarsurengin et al., 2003), (Gao et al., 2004)
APAF-1	Apoptotic protease activating factor	?	Melanoma, neuroblastoma	(Bunz, 2001)
RIZ/PRDM2	Histone/protein methyltransferases	Familial colorectal cancer	Colorectal, gastric, endometrial, pancreatic cancers	(Canote et al., 2002), (Derunes et al., 2005), (Geli et al., 2005)
PinX1	Telomerase regulatory protein	Prostate cancer	Hepatocellular carcinoma, liver, lung, colorectum, breast cancers	(Zhou and Lu, 2001)
NPRL2/G21	Mismatch repair, cell cycle checkpoint signaling, and activation of apoptotic pathways	?	Lung, breast, cervical, oral cavity, ovary, kidney	(Li et al., 2004)
ING1	Inhibitor of growth	?	Melanoma, breast cancer	(Nouman et al., 2003), (Cheung and Li, 2001)

? 아직 정확한 보고가 없음 .

그 중에서 가장 큰 역할을 하는 것이 cyclin dependent kinase (CDK)/cyclin complexes이다. 또한 CDK/cyclin complexes을 조절하여 정상적인 세포주기가 유지되도록 역할을 담당하는 $p16^{INK4a}$, $p21^{Cip1/WAF1}$, $p27^{KIP1}$, p57 등과 같은 cyclin dependent kinase inhibitor (CKI) 들이 있다 (Jacks, 1996; Sherr and Roberts, 1995). 이들 CKI들은 넓은 의미로 tumor suppressor gene (TSG)에 속한다 (Table 1) (Jacks, 1996; Sherr, 2004).

정상세포의 경우에 세포주기의 시작점인 G1 phase에서는 CDK4 또는 CDK6와 cyclin D complexes가 주로 관여하는데, transcription factor인 E2F와 complex를 이루는 Retinoblastoma (Rb) protein을 인산화 (phosphorylation) 시켜서 E2F에서 떨어져 나가게 한다. 이때 분리된 E2F는 다음 세포주기 단계에서 필요한 DNA polymerase-α(POL), CDC2, cyclinE/A, E2F-1 자신뿐만 아니라 dihydrofolate reductase (DHFR), thymidine kinase (TK), thymidylate synthase (TS) 등의 유전자 발현을 개시한다 (Sherr, 1994; Sherr, 1996).

G1 phase에는 restriction point가 존재하여 세포 내 환경이 다음 단계인 S phase로 가기에 적합한지의 여부가 결정되어 일단 이 지점을 통과하게 되면 growth factors에 independent 하게 세포주기가 다음 단계로 계속 진행된다. G1 phase를 조절하는 tumor suppressor gene으로 대표적인 것이 $p16^{INK4a}$ (CDKN2A, MTS1, CDK4I)이다 (Sherr, 1996).

일반적으로 human tumorigenesis는 p16의 기능소실 (functional activity)로 인한 p16/CDK4/cyclinD/Rb pathway의 작동불능 때문이다 (Liggett and Sidransky, 1998).

p16이 functional activity를 잃게 되는 원인으로는 mutation, deletion, promoter methylation등과 같은 변성 (alteration) 때문이며, 이로 인하여 astrocytoma, melanoma, leukemia, breast cancer, head and neck squamous cell carcinoma, malignant mesothelioma, osteosarcoma, bladder cancer, ovarian cancer, lung cancer, liver cancer, renal cancer 등의 다양한 종류의 암이 유발된다 (Table 2) (Rocco and Sidransky, 2001). 또한 배양된 여러 암세포 주에서도 유사한 p16의 변성을 볼 수 있다 (Table 3) (Kamb *et al.*, 1994; Kubo *et al.*, 1999; Okamoto *et al.*, 1994).

INK4 family proteins

INK4는 specific polypeptide inhibitors of cyclin dependent kinase 4 에서 유래한 일련의 단백질들을 칭한다 (Sherr, 1996). 이들 INK4 family는 chromosome 9q21의 genomic locus를 공유하고 있으며, $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$, $p19^{INK4d}$ (in human $p14^{ARF}$)이 여기에 속하며, 각각의 molecular weight에 따라서 이름이 명명되었다 (Fig. 2). p15와 p16 protein의 염기서열은 약 80% 정도 동일성 (identity)을 보이고, p18과 p19에 대해서는 약 40~45%의 동일성을 보인다. 그들의 염기서열이나 구조들은 매우 유사하지만 conformational flexibility, stability, aggregation 경향에 따라서 약간씩 차이가 남을 보인다 (Yuan *et al.*, 1999). 가장 많이 보존 되어있는 1차 염기서열은 helical regions이고 아마도 이 부분들이 앞에 언급한 INK4 family 단백질들의 3차 구조상의 유사성을 가지게 하는 것으로 여겨진다. $p15^{INK4b}$와 $p16^{INK4a}$은 네 개의 helix-turn-helix motifs를 가지고 p18과 p19는 다섯 개의 helix-turn-helix motif를 hydrophobic interaction을 통해서 갖는다 (Li *et al.*, 1999; Yuan *et al.*, 1999).

$p16^{INK4a}$를 encoding하는 locus는 서로 다른 promoters로부터 두 개의 transcripts를 만들어 낼 수 있다. 각각의 transcript는 specific 5' exon E1α 나 E1β을 가지는데 이들이 splice 되어서 exon E2, E3가 된다. E1α를 포함하는 transcript가 $p16^{INK4a}$을 encode하고 E1β를 포함하는 transcript가 $p14^{ARF}$를 encode한다 (Duro *et al.*, 1995). $p14^{ARF}$는 MDM2와 binding하여 MDM2-mediated Rb inactivation과 MDM2-mediated p53 degradation을 저해함으로써 직접적으로 CDK/cyclin complex를 억제하기보다는 간접적인 경로를 통해서 G1과 G2 phase에서 세포의 성장을 억제한다 (Bates *et al.*, 1998; Stott *et al.*, 1998). p15는

Table 2. Frequency of $p16^{INK4A}$ inactivation

Cancer type	Number of studies	Positive cases (%)	Reference
Prostate carcinoma	118	91(77.1)	(Jeronimo et al., 2004)
Gastric tumor	81	16(20)	(An et al., 2005)
Gastrointestinal stromal tumor	21	7(33.3)	(Ricci et al., 2004)
Lung cancer	91	14(15.4)	(Fujiwara et al., 2005)
Non-small-cell lung cancer	351	86(25)	(Toyooka et al., 2004)
Renal cancer	17	6(35)	(Hoque et al., 2004)
Primary colorectal carcinomas	53	17(32)	(Lind et al., 2004)
Squamous cell carcinoma	36	13(36)	(Brown et al., 2004)

Table 3. Status of $p16^{INK4A}$ on tumor cell lines

Cell lines	Alteration	Cell lines	Alteration	Cell lines	Alteration	Cell lines	Alteration
Skin		Lung		Colon		Ovary	
LOX IMVI	HD	A549	HD	COLO205	TD	IGROV1	W
M14	PM	BEAS2B	W	DLD-1	M	OVCAR-3	W
MALME-3M	HD	866MT	M	HCC-2998	W	OVCAR-4	W
SK-MEL-2	W	Calu-1	ND	HCT-15	TD	OVCAR-5	HD
SK-MEL-5	HD	Calu-6	ND	HCT-116	PM	OVCAR-8	W
SK-MEL-28	W	EKVX	TD	HT-29	TD	SK-OV-3	HD
UACC-62	HD	HOP-62	HD	LS174T	TD	Breast	
UACC-257	PM	HOP-92	HD	SK12	TD	MCF-7	HD
Lymphocyte		NCI-H23	TD	SW403	TD	MDA-MB-231	HD
CCRF-CEM	HD	NCI-157	M	SW620	TD	HS 578T	HD
HL-60	PM	NCI-H226	HD	Renal		MDA-MB-435	PM
K-562	HD	NCI-322(M)	HD	SN12C	W	MDA-MB-N	PM
MOLT-4	HD	NCI-H358	ND	TK-10	TD	T-47D	TD
RPMI-8226	TD	NCI-H460	HD	786-0	HD	MCF/ADR-RES	W
SR	HD	NCI-H522	W	A498	HD	BT-549	W
Liver		NCI-H596	W	ACHN	HD	CNS	
THLE5B	W	NCI-N417	W	CAKI-1	HD	SF-268	HD
Hep3B	W	Pancreas		RXF-393	HD	SF-295	HD
HepG2	W	ASPC-1	ND	UO-31	HD	SF-539	W
HuH4	ND			Prostate		SNB-19	HD
Ha22T/VGH	M			DU-145	PM	SNB-75	W
HB611	W			PC-3	TD	U251	HD

W: wild type, PM: point mutation, HD: homozygous deletion, TD: transcriptional deficiency, ND: no detected

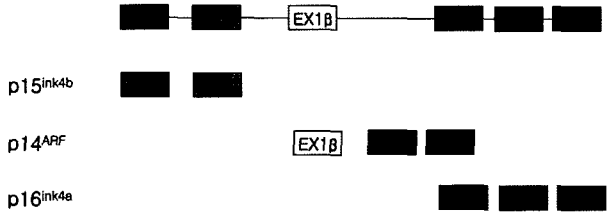

Fig. 2. 9p21 locus.

transforming growth factor (TGF)β에 의해 유도되는 세포주기억제(cell-cycle arrest)에 중요한 역할을 할 것으로 생각된다 (Hannon and Beach, 1994).

Function of p16

Rb pathways

세포주기는 CDK들의 sequential activation과 inactivation에 의해 조절된다(Hunter and Pines, 1994; Serrano *et al.*, 1996). 각각의 CDK는 catalytic activity와 substrate recognition에 필수적인 역할을 하는 cyclin subunit과 결합하고 있다. cyclin family 중에서 cyclin D1은 oncogene 또는 세포주기의 G1 phase에서 mitogen에 의해 유도되는 성장 활성인자로써 밝혀졌다. Cyclin D1에 의해 활성화되는 주요 CDK partner는 CDK4와 CDK6이다. 이들 kinases의 activity는 inhibitory subunit에 의하여 조절된다. 이런 inhibitor중에 하나가 $p16^{INK4a}$인데, $p16^{INK4a}$는 human cells에서 CDK4-associated protein으로써 처음 알려졌다 (Xiong *et al.*, 1993). 그 후에 클로닝이 되었고 CDK4-6/cyclinD complexes에 대한 specific inhibitor로써 characterize되었다 (Serrano *et al.*, 1993). *in vivo*에서 CDK4-6/cyclinD kinases의 결정적인 기질은 retinoblastoma-susceptibility tumor suppressor protein (Rb)이다. Rb는 G1 phase에서 S phase로 넘어가는데 필요한 transcription factor인 E2F를 일련의 과정을 통해서 negative하게 조절한다. Transcription factor에 결합하는 Rb의 기능은 G1 phase의 kinase에 의해서 유도되는 인산화 (phosphorylation)에 의해서 저해 되는데 mitosis과정이 끝날 때까지 지속된다.

CDK4/6 inhibitor인 $p16^{INK4a}$이 overexpression된 경우에, p16이 CDK4/6의 regulatory domain에 결합하여 conformational change를 일으켜 cyclin D와의 complex 형성을 저해한다. 이때 Rb가 인산화되지 못하고 E2F가 불활성화 됨으로써 전사가 저해되어 G1 phase에서 cell cycle이 arrest 된다 (Russo *et al.*, 1998).

NF-kB pathways

Nuclear factor *k*B (NF-*k*B)는 면역 및 염증반응뿐만 아니라 세포의 성장 (growth), 분화 (differentiation), 사멸 (apoptosis)등 세포주기 조절에 있어서 중요한 역할을 하는 유전자들의 발현을 조절하는 전사인자이다 (Baeuerle and Baichwal, 1997; Baldwin *et al.*, 1991; Bash *et al.*, 1997; Chen *et al.*, 2001; Duyao *et al.*, 1990; Guttridge *et al.*, 1999; Hinz *et al.*, 1999; Mercurio and Manning, 1999; Perkins, 2000). 불활성 상태에서는 cytoplasm에서 inhibitory protein인 I*k*B와 결합되어 있다가 세포가 특정 inducers에 의해 stimulation 되면 I*k*B kinase (IKK)에 의해서 I*k*B가 인산화되어 ubiquitinylation에 의해 degradation되고 p50, p65로 이루어진 NF-*k*B complex가 핵 내로 이동하여 *k*B DNA binding domain에 결합하여 표적유전자의 전사를 유도한다. NF-*k*B의 비정상적인 과도한 전사활성으로 인해 암이 발생 되어진다는 여러 보고가 있으며 (Dhawan *et al.*, 2002; Gilmore *et al.*, 1996; Luque and Gelinas, 1997), NF-*k*B 전사활성과 CDK의 조절 관계는 Gary J. Nabel에 의해 처음 보고되었다 (Perkins *et al.*, 1997).

INK4 단백질의 ankyrin repeats는 protein-protein interaction motif로써 잘 알려져 있으며 NF-*k*B inhibitor인 I*k*B family에 있어서도 중요한 motif로써 알려져 있다 (Sedgwick and Smerdon, 1999). human cell lines에서 많은 양의 INK4 molecule들이 존재하고 있다는 것으로 추정해 볼 때 잘 알려진 표적인 CDK4/CDK6 이외의 다른 표적 단백질들이 존재 할 가능성을 생각해 볼 수 있는데, Wolff와 Naumann이 INK4 proteins이 NF-*k*B를 표적으로 하여 NF-*k*B의 활성을 저해 함을 밝혔다 (Wolff and Naumann, 1999). 실제로 melanocytic lesions에서 16^{INK4a} alteration에 의한 발현 감소는 NF-*k*B p65이 모반 (nevi), 일차 흑색종 (primary melanomas), 전이 (metastases)로 감에 따라서 발현이 증가됨을 보였다 (Ghiorzo *et al.*, 2004). 그리고 ankyrin repeats를 통한 p16과 I*k*Bα에 대한 human T cell leukemia virus I (HTLV-1) tax oncoprotein의 상호작용은 p16이 CDK4를, I*k*Bα가 NF-*k*B를 저해하는 능력을 잃게 하였다 (Hirai *et al.*, 1994).

p16 in cancer

Tumor susceptibility of $INK4a^{-/-}$ mice

Tumor suppression에 있어서 INK4a의 역할을 규명하기 위해서 carcinogen을 처리한 군과 처리 하지 않은 군을 heterozygous intercrosses하여 tumor formation 정도를 측정 해 보았다 (Serrano *et al.*, 1996). un-treated $INK4a^{-/-}$ mice 중 69%가 평균 29주에서 조직학적으로 양성인 종양이 형성되었다. 반면 $INK4a^{+/+}$나 $INK4a^{+/-}$중에서는 36주가 지나서도 종양이 확인되지 않았다. 또한 $INK4a^{-/-}$, $INK4a^{+/-}$, $INK4a^{+/+}$의 genotype들에 대해서 9,10-dimethyl-1,2-benzanthracene (DMBA)를 한번 처리한 후 ultraviolet B (UVB) (280–320 nm)를 여러 번 조사한 경우, DMBA alone 처리한 경우, UVB만 조사한 경우로 서로 다른 세가지 처리방법을 이용하여 tumor induction을 확인하였다. 20주 동안 관찰했을 때, $INK4a^{-/-}$ mice군의 약 90%가 DMBA/UVB를 처리한 경우에 tumors가 형성되거나 건강상태가 좋지 않은 것으로 나타났다. 특히 20마리의 $INK4a^{-/-}$ mice 중에서 12마리가 조직학적으로 명백한 malignancy를 나타내었으며 평균적으로 9주정도가 되었을 때 tumors가 형성되었다. 반면 23마리의 $INK4a^{+/-}$ 중에 83%, 27마리의 $INK4a^{+/+}$ 중에 100%가 건강한 상태로 남아있었다. 이러한 결과들로 비추어 볼 때 INK4a가 결여되었을 때 암의 발생에 대한 민감도 (susceptibility)가 증가함을 알 수 있다.

p16 mutations and deletion

9p21 locus는 일반적으로 human tumor에서 amino acid mutations 이나 protein을 인코딩하는 exon들에 영향을 미쳐 premature termination됨으로써 distruption된다 (Kamb, 1995; Kamb *et al.*, 1994; Nobori *et al.*, 1994; Sherr, 1995). Table 2에 나와 있는 바와 같이 p16 locus의 mutation 이나 deletion이 primary cancers에서 15% 이상 발견됨을 알 수 있다. Biliary tract에서는 약 50%, esophageal carcinoma에서는 약 30%이 p16의 mutation에 의해서 일어나고 (Elledge and Harper, 1994; Hall and Peters, 1996) glioma와 esothelioma에서는 각각 약 50%, nasopharyngeal carcinoma에서 약 40%, acute lymphocytic leukemia에서 약 30%가 INK4a locus의 homozygous deletions이 관찰되며, sarcomas, bladder, ovarian tumors 등에서도 관찰된다. 또한 pancreatic, head and neck, non-small-cell lung carcinoma의 경우는 INK4a의 mutations과 deletions이 관여함이 보고되었다 (Hall and Peters, 1996). p16의 tumor specific alteration은 protein function에 영향을 미쳐서 cdk4/6와 interaction을 불안정하게 함으로써 p16의 inhibitory activity를 감소시

킨다. 예로써, p16의 tumor-derived mutation으로 114번째의 proline이 leucine으로 바뀌었을 때 in vitro에서 cdk4와 binding 하지 않으며 이로인해 G1 arrest도 유도하지 못하였다.

p16 methylation

DNA methylation은 포유류의 경우에 후생적인 (epigenetic) 현상이다 (Jones and Baylin, 2002). 특히 post-replication modification에서 이루어지며 dinucleotide sequence CpG에 존재하는 cytosine residues에서 발견된다. Cancer cell에서는 gene silencing의 의미로 쓰인다. Tumor suppressor genes의 promoter region에 위치한 CpG island의 hypermethylation은 gene inactivation에 대한 중요한 메커니즘 중에 하나이다. $p16^{INK4a}$의 hypermethylation은 colorectal, lung, pancreas와 같은 많은 solid tumor types에서 발견될 뿐만 아니라 bladder, cervical tumors, melanoma, glioma에서도 epigenetic silencing이 일어난다. 91명의 Lung cancer환자의 serum DNA에서 약 15.4%가 methylation되었으며, 그 중 clinical stage I에서 13.2%, III에서 22.7%, IV에서 22.2%의 빈도를 나타내었다 (Fujiwara *et al.*, 2005). 57명의 pulmonary adenocarcinoma 환자의 tumor sample 중에 40.4%인 23명이 abberrent methylation을 나타내었고 (Tanaka *et al.*, 2005), cutaneous squamous cell carcinoma (SCC)에서는 36명 중에 약 36% (Brown *et al.*, 2004), gastrointestinal stromal tumor에서는 21명중에서 약 33.3% (Ricci *et al.*, 2004), prostate carcinoma에서는 118명중에 77.1% (Jarrard *et al.*, 1997), primary colorectal carcinoma에서는 32% (Lind *et al.*, 2004)가 promoter methylation이 일어났다. 반면에 chronic myelogenous leukemia (Kusy *et al.*, 2004), breast fibroadenoma/carcinoma (Di Vinci *et al.*, 2005), multiple myeloma에서는 biological significance를 찾지 못하였다 (Jeronimo *et al.*, 2004).

p16 in cancer therapy

p16의 tumor suppressor 기능은 항암치료의 전략에 활용되고 있다. pRB을 정상적으로 발현하지만 p16이 mutation되었거나 null되어진 breast cancer cell line (MCF-7, MDA-MB-231, BT-549)과 osteosarcoma cell line (U-2 OS)에 recombinant adnovirus Adp16을 infection하여 p16의 기능을 회복시켰을 때 대조군에 비하여 cell cytotoxicity가 3배 이상 증가하였다 (Craig *et al.*, 1998). pRB 인산화를 저해하기 위하여 p16와 CDK4/6의 interaction하는 주요 아미노산 잔기인 84번에서 103번의 약20개의 합성 펩타이드를 antennapedia carrier를 이용하여 human HaCaT cells에 처리하였을때, S-phase로의 진행이 차단되었고 (Fahraeus *et al.*, 1998; Fahraeus *et al.*, 1996), Trojan p16 peptide를 이용하여 in vitro 및 in vivo에서 pancreatic cancer growth가 저해되는 것이 확인되었다 (Fujimoto *et al.*, 2000; Hosotani *et al.*, 2002). 또한 p16-/pRB+인 A375M 흑색종 세포에서도 p16-mimicking peptide를 처리시 종양 크기가 줄어 들었다 (Noonan *et al.*, 2005). p16과 p27의 fusion tumor suppressor gene (AV-W9)을 adenoviral system에 적용시켜 과발현시킨 prostate, lung, colon, breast, retina 등의 14가지 cancer cell line중 12개에서는 apoptosis 유도를 통한 tumor 저해가 일어났으나, 나머지는 cell death, growth arrest를 일으켰다 (Patel *et al.*, 2000). p16의 N-terminal쪽 40개 아미노산이 cell transformation을 유도하는 JNK와 상호작용하여 Ras-JNK-c-Jun-AP-1을 축으로 하는 signal pathway의 활성을 저해함으로써 UV에 의해서 유도되는 SK-MEL-28 흑색종 세포의 성장이 억제됨을 보고하였다 (Choi *et al.*, 2005).

Perspective

$p16^{INK4a}$는 세포 주기 중에서 G1 phase에 관여하여 CDK4/cyclinD complex의 activity를 저해함으로써 세포성장을 조절하며, $p15^{INK4b}$, $p18^{INK4c}$, $p19^{INK4d}$과 함께 INK4 family에 속한다. 암의 경우에 tumor suppressor gene인 p16의 gene mutation, deletion, promoter region의 methylation 등의 변성으로 인해서 세포의 성장과 억제에 대한 균형이 깨지게 되면 암을 유발할 수 있으며, UV나 세포성장인자 등에 대한 susceptibility가 증가하게 된다. 이러한 p16의 기능을 이용하여 p16이 결여된 selected cancer에 p16 peptide gene을 도입 하였을 때 치료효과를 나타내었고, 다른 gene과 combination을 시킨다면 broad spectrum의 cancer therapy에 상당한 효과를 기대할 수 있을 것이다. 또한 p16이 가지고 있는 구조적 특징인 ankyrin repeat motif는 p16이 CDK4/cyclinD complex뿐만 아니라 NF-*k*B complex와 JNK에도 영향을 미치는 것으로 보아 암 발생을 유도하는 또 다른 주요 단백질의 기능을 조절하는 p16의 multifunction에 대한 연구를 기대해 볼 수 있을 것이다.

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