분자역학의 독성학적 이해

AITOX

‘삼성전자 백혈병’ 박지연씨 끝내 숨져

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삼성반도체 온양공장에 입사했다가 급성 골수성백혈병에 걸려 32개월 간 투병해 온 박지연(23) 씨가 지난 31일 사망했다.

삼성백혈병충남대책위는 삼성전자 온양공장에서 일하던 중 급성골수백혈병에 걸려 투병하던 박 씨가 31일 오전 11시께 숨졌다고 1일 밝혔다.

고 박지연 씨는 2004년 12월 입사해 2년8개월간 검사과에서 일하다 백혈병에 걸렸고 긴 투병 끝에 세상을 떠났다.

반도체 노동자의 건강과 인권을 생각하는 모임(반올림)은 “삼성반도체 온양공장은 기흥공장에서 가공된 웨이퍼를 절단·조립·검사하여 반도체 완제품을 만드는 공장으로 최소 22명 이상이 백혈병에 걸렸고, 최소 9명이 사망했다”고 주장했다.

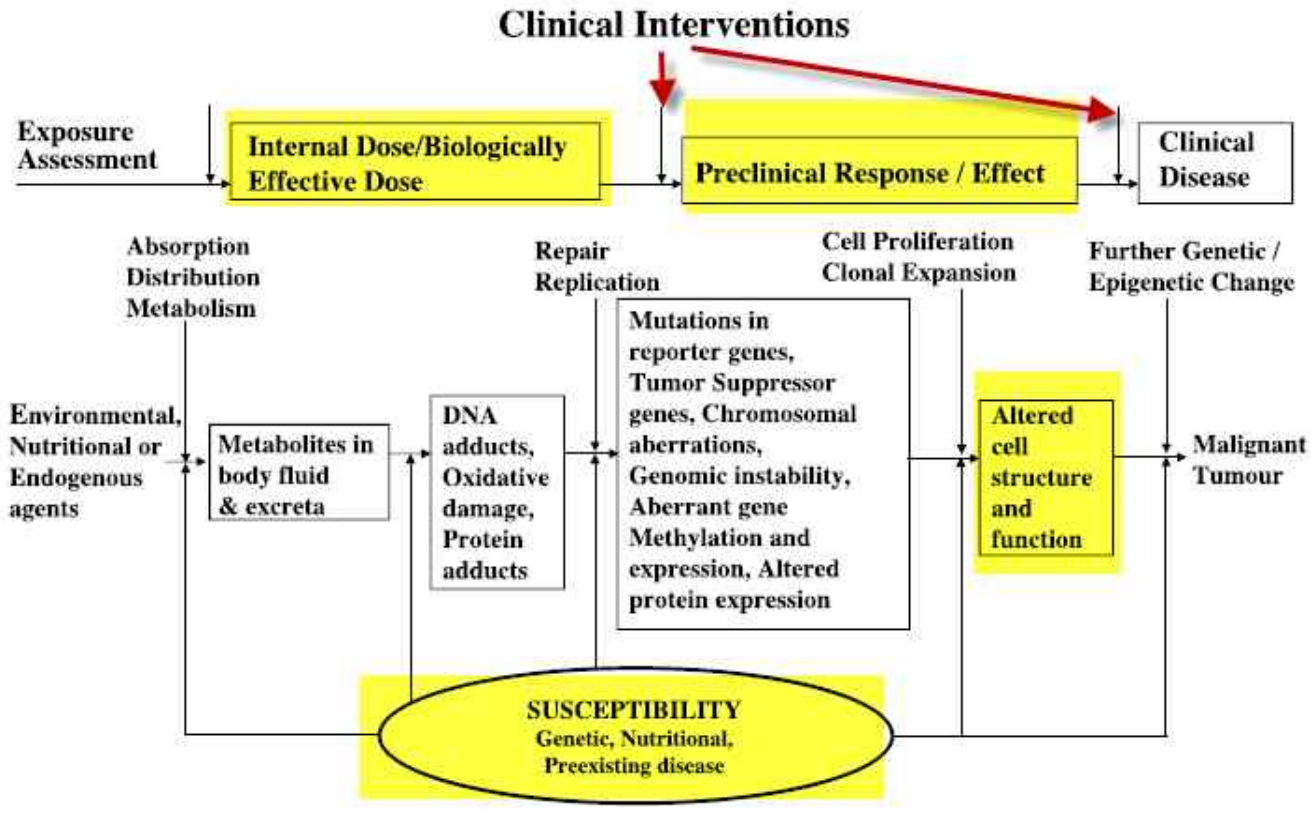
1. 분자역학<Molecular epidemiology>의 기원과 개념

○ 1982년: Perera과 Weinstein: ‘‘Molecular cancer epidemiology’’: 원인인자와 질환 발생의 이론적 전체과정(continuum)에서 기전과 변화를 biomarker을 이용하여 발암의 역학적 연구에 대입 – 발암 연구의 새로운 paradigm을 제시

※ Molecular epidemiology 출현의 배경논문: Perera FP, Weinstein IB. Molecular epidemiology and carcinogen DNA adduct detection: new approaches to studies of human cancer causation. J Chronic Dis 1982;35:581-600.

○ 1987년: U.S. National Academy of Sciences: Biomarker를 3개 영역인 makers of internal dose, markers of biologically effective dose, markers of early response/effect와 markers of susceptibility의 분류에 “altered structure and function”를 포함하여 biomarker의 4개 영역으로 <그림>의 The origin model of “Molecular Epidemiology과 같이 Perera과 Weinstein의 paradigm를 upgrade.

○ The origin model of “Molecular Epidemiology: Biomarker의 발암의 역학적 연구 도입하여 예방 및 치료를 위한 단계별 접근



○ Molecular epidemiology: Biomarker-based epidemiology

2. 분자역학에서의 독성학적 이해(Toxicological approaches)의 목적과 방법

○ Biomarker is anything that can be used as an indicator of a particular state or progress of a disease.

○ **Biomarker requirements :** Timely , Easily(non-invasive), Specificity and Sensitivity

○ The origin model of "Molecular Epidemiology"에서 4개 분야의 Biomarkers: Biomarkers of internal dose, Biomarkers of biologically effective dose, Biomarkers of early response/effect와 Biomarkers of susceptibility

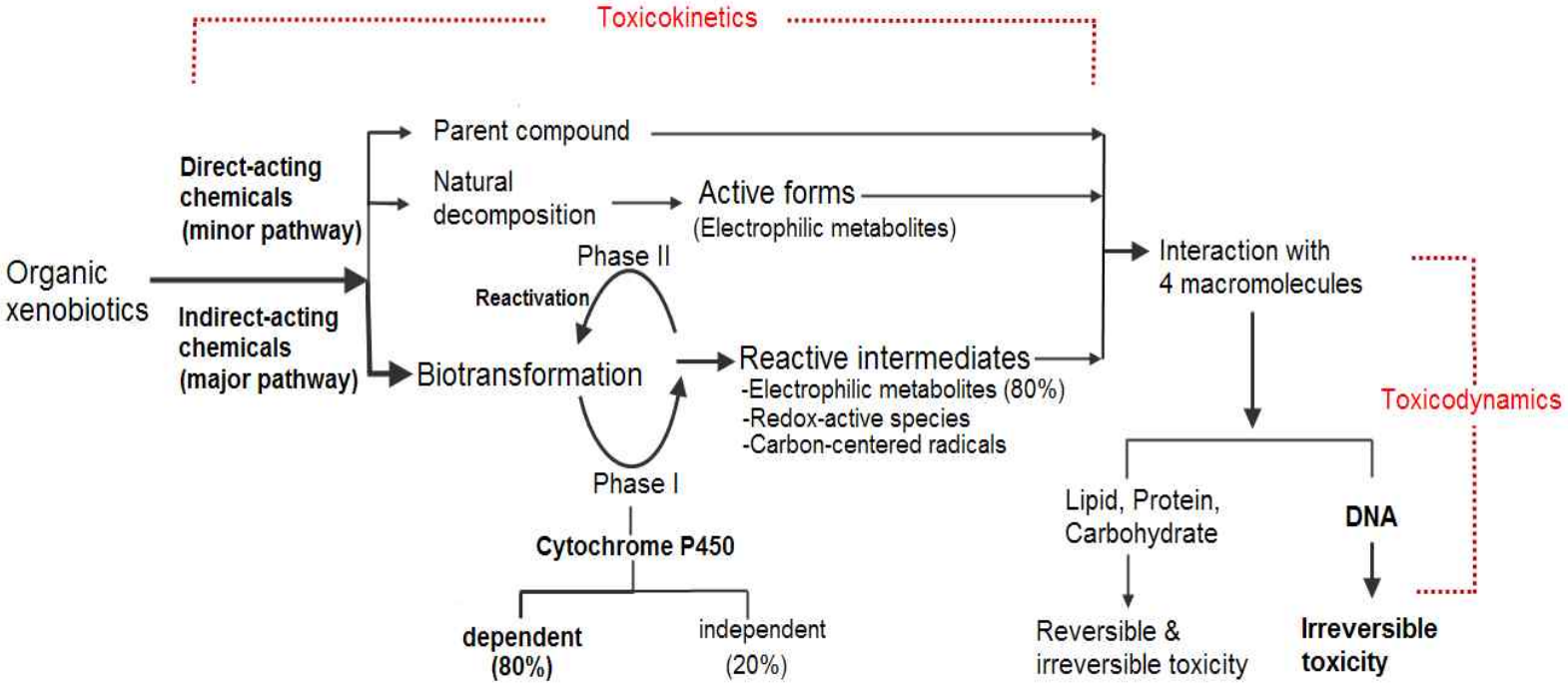
○ 본 연구에서는 **Benzene과 Acute Myelogenous Leukemia(AML,** 급성 골수성 백혈병**)**를 The origin model of "Molecular Epidemiology"에 대입하여 **Biomarker requirement에 얼마나 부합하는가를 확인하는 것이** 목적이다.

○ **Benzene**은 독성학적으로 가장 많이 연구된 물질이므로 결국 이를 통해 독성물질의 노출에 대한 발암과정에서 biomarker가 얼마나 개발되었는가 하는 현주소를 확인하는 것이다.

3. Benzene과 leukemia에 대한 molecular epidemiology

1) 독성학의 기본 개념

○ 유기물질의 "독성학의 시작과 끝은 toxicokinetic와 toxicokinetics"이라는 개념으로 "Central dogma" 개발

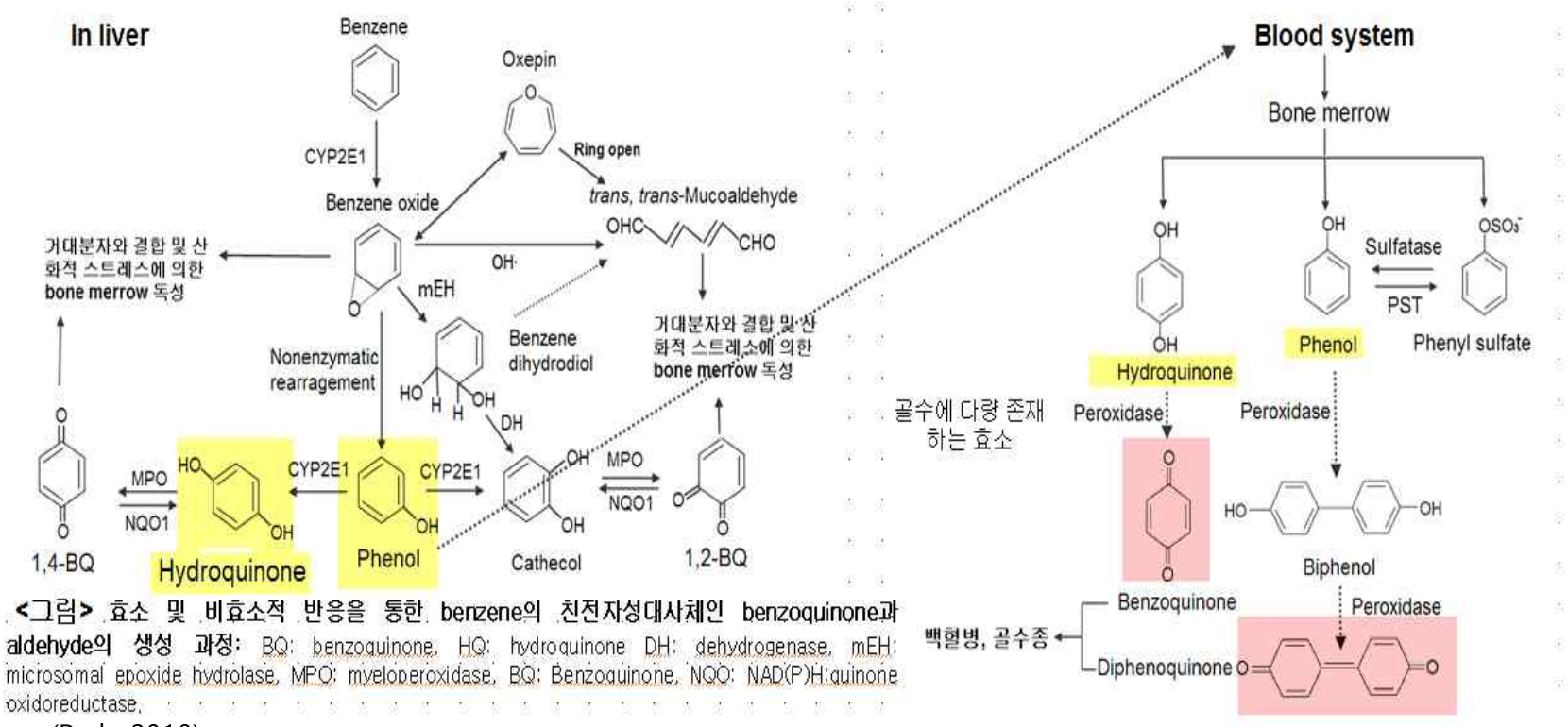


(Park, 2010)

1) Benzene에 의한 leukemia 발생 기전

○ 두 가지 독성기전: 대사체 측면(Diphenoquinone, Benzoquinone, hydroquinone)과 ROS(reactive oxygen species)

① 대사체 측면(Diphenoquinone, Benzoquinone)



<그림> 효소 및 비효소적 반응을 통한 benzene의 친전자성대사체인 benzoquinone과 aldehyde의 생성 과정: BQ: benzoquinone, HQ: hydroquinone DH: dehydrogenase, mEH: microsomal epoxide hydrolase, MPO: myeloperoxidase, BQ: Benzoquinone, NQO: NAD(P)H:quinone oxidoreductase.

(Park, 2010)

② ROS 측면

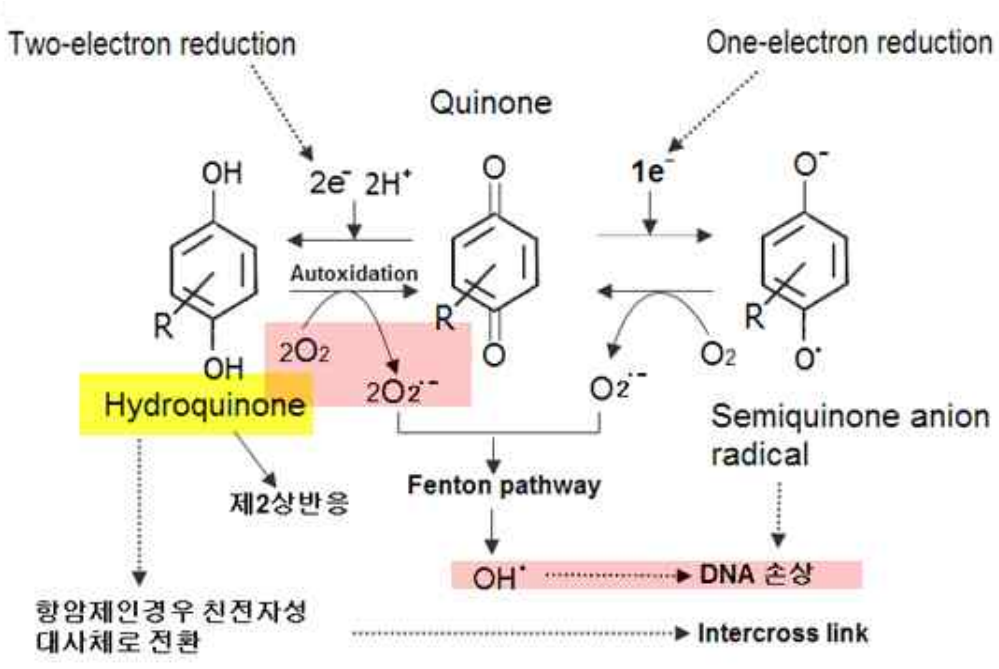


그림-16: Quinone 화합물의 one-electron reduction과 two-electron reduction: One-electron reduction(일전자-환원)은 cytochrome P450 reductase 등과 같은 효소의 촉매작용으로 semiquinone이 형성되면서 세포에 직간접적인 독성을 유발한다. 그러나 NQO1 등에 의한 이전자-환원(two-electron reduction)은 hydroquione을 생성하면서 제2상반응을 통해 체외로 배출되는 무독화 과정이다. 그러나 hydroquinone은 자동산화(autoxidation)을 통해 다시 quinone을 산화되는 redox cycle을 반복하며 ROS를 생성하기도 한다. (참고: Bolton)

(Park, 2010)

3. Benzene과 leukemia에 대한 molecular epidemiology

2) Biomarker and leukemia based on the model

①Biomarker of internal dose after benzene exposure: Urinary Benzene(UB)가 대사체보다 internal dose를 잘 반영

Table I. Summary of benzene exposures and UB levels in workers occupationally exposed to benzene and controls in Shanghai, China

Group	Parameter	Benzene exposure (p.p.m.)	UB (μg/l)
Control	Mean ± SD	0.015 ± 0.018	0.145 ± 0.335
	Median (range)	0.016 (0–0.11)	0.069 (0.027–2.06)
	No. of workers	41	41
	No. of smokers	18	18
Lower exposure (≤31 p.p.m.)	Mean ± SD	14.5 ± 8.96	8.42 ± 9.0
	Median (range)	13.6 (1.65–30.6)	4.95 (0.837–27.9)
	No. of workers	22	22
	No. of smokers	12	12
Higher exposure (>31 p.p.m.)	Mean ± SD	112 ± 76	50.2 ± 62.5
	Median (range)	92.0 (31.5–329)	46.1 (1.30–284)
	No. of workers	20	20
	No. of smokers	7	7

(Waidyanatha et al., 2001)

Table II. Least squares regression of urinary metabolites of benzene[a] on UB[b] among workers occupationally exposed to benzene and matching controls in Shanghai, China

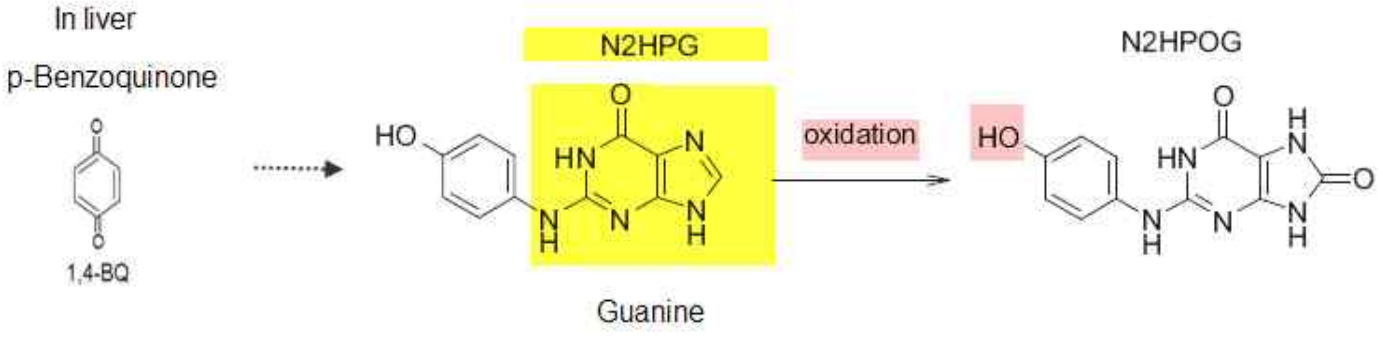
Metabolite	Relationship[d] (Pearson *r*)	
	Exposed subjects	All subjects
Phenol	ln(phenol) = 3.2 + 0.60 ln(UB) (0.815)	ln(phenol) = 3.7 + 0.42 ln(UB) (0.826)
Catechol	ln(catechol) = 1.4 + 0.62 ln(UB) (0.815)	ln(catechol) = 2.0 + 0.39 ln(UB) (0.812)
Hydroquinone	ln(hydroquinone) = 2.1 + 0.49 ln(UB) (0.736)	ln(hydroquinone) = 2.1 + 0.48 ln(UB) (0.898)
Muconic acid	ln(MA) = 1.7 + 0.54 ln(UB) (0.730)	ln(MA) = 1.0 + 0.79 ln(UB) (0.938)

[a]Hydroquinone, phenol and MA levels were available for 43 exposed workers and 17 controls; catechol levels were available for 42 exposed workers and 16

② Biomarker of biological effective dose (DNA adduct Before cell division)

○ N2HPG(N2-4-hydroxyphenyl guanine)을 rat에 투여 후 약 8%가 urine으로 24시간이내 배출

○ 중요한 점은 non-invasive 방법으로 Benzene-DNA adduct가 biological effective dose 확인을 위한 biomaeker로써의 가능성



Scheme 1. Urinary metabolites of N2HPG and routes of their formation.

Table 1
Excretion of N2HPG and its metabolite, N2HPOG, in the urine of rats dosed with a single *ip* injection of 2 mg/kg N2HPG.

Time interval [h]	Urinary excretion [% of dose; mean ± SE, $n = 3$]		
	Untreated urine		
	N2HPG	N2HPOG	Total[a]
0–24	8.0 ± 2.2	2.7 ± 0.2	10.7 ± 2.7
24–48	1.2 ± 0.6	<0.2[b]	1.2 ± 0.6
0–48	9.1 ± 1.7	2.7 ± 0.2	11.8 ± 2.1

[a] N2HPG + N2HPOG.
[b] N2HPOG was not detected. The limit of detection 100 ng/mL corresponds to approximately 0.2% of the dose.

(Linhart et al., 2011)

③ Biomarker of early response/effect (Mutation after cell division)

○ **The Nature of Chromosomal Aberrations Detected in Humans Exposed to Benzene** (Zhang et al., 2002): 수 많은 mutation 종류 중에 Benzene-specific pattern의 mutation이 없다.

TABLE 12
Prevelance of Specific Chromosomal Aberrations in Leukemia Patients with Likely Prior Exposure to Benzene[a]

Chromosomal Aberration	Case Number	Rate (%) (n=41, abnormal only)[b]	Rate (%) (n=59, All)[c]
Translocations	**22**	**54 (22/41)**	**37 (22/59)**
t(21q22)	7	17	12
t(8;21)	5	12	8
t(9;22)	4	10	7
t(15;17)	2	5	3
t(12q)	4	10	7
t(17p)	2	5	3
Deletions	**14**	**34**	**24**
del(11q)	2	5	3
del(12p)	2	5	3
del(17p)	2	5	3
-5/5q- & -7/7q-	**17**	**41**	**29**
-5/5q-	9	22	15
-7/7q-	12	29	20
5q-/7q-	**11**	**27**	**19**
5q-/t(5q)	8	20	14
5q-	5	12	8
t(5q)	3	7	5
7q-	3	7	5
-5/-7	**11**	**27**	**19**
-5	2	5	3
-7	9	22	15
Aneuploidy	**29**	**71**	**49**
±C	19	46	32
+C	6	15	10
-C	14	34	24
±8	7 (2/5)	17	12
±21	7 (3/4)	17	12
-17/-18	6	15	10
±Y	6	15	10

[a] The most frequent abnormal karyotypes shown in Table 2 are summarized into each chromosomal aberration category.
[b] The frequency of specific chromosome changes in the total of 41 patients with abnormal karyotypes only.
[c] The frequency of specific chromosome changes in the total of 59 patients with normal and abnormal karyotypes.

③ Biomarker of early response/effect (Mutation after cell division)

○ Case-control study에서 chromosome 7 monosomy: 노출용량-의존적 유의성

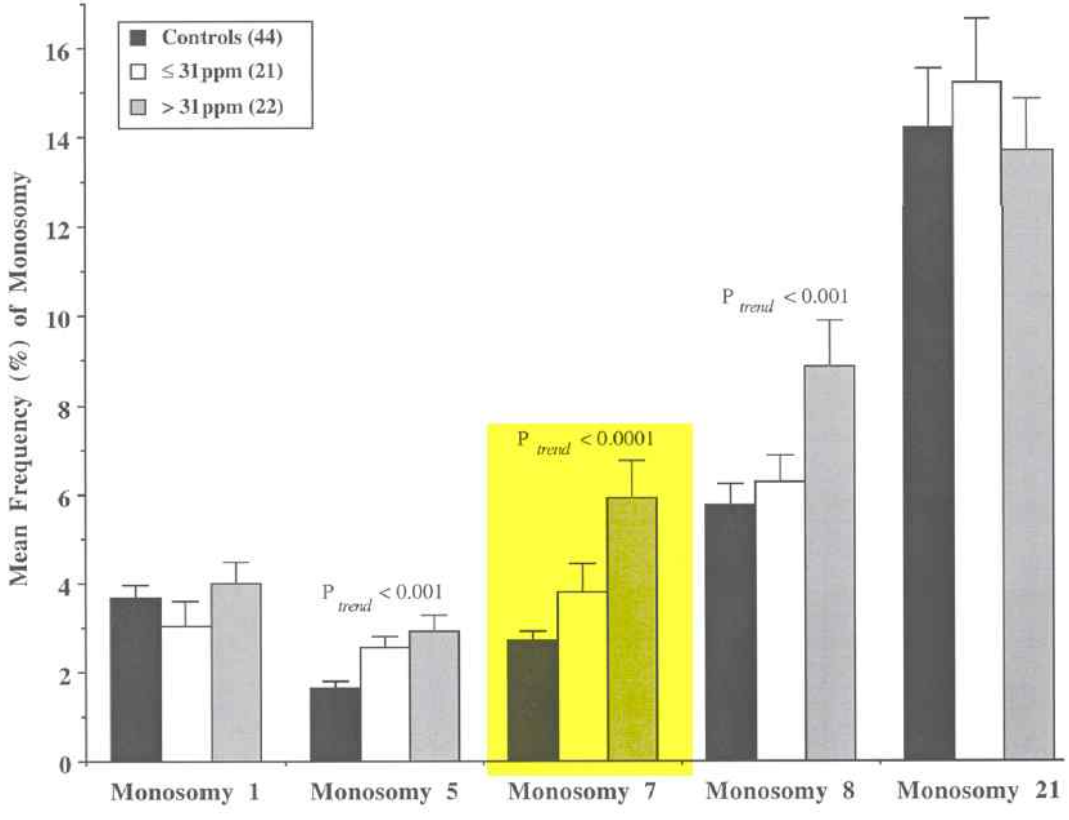


FIGURE 2. Monosomy of chromosomes 1, 5, 7, 8, and 21 in workers exposed to benzene and controls. Data presented are mean monosomy frequency (%). Error bar represents S.E. Black bar represents control, white bar represents ≤ 31 ppm group, and gray bar represents > 31 ppm group. Test for trend was performed by linear regression on square-root transformed data.

(Zhang et al., 2002)

○ Hdyroquinone 대사체에 의한 chromosome 7 monosomy 가장 높은 발생확인

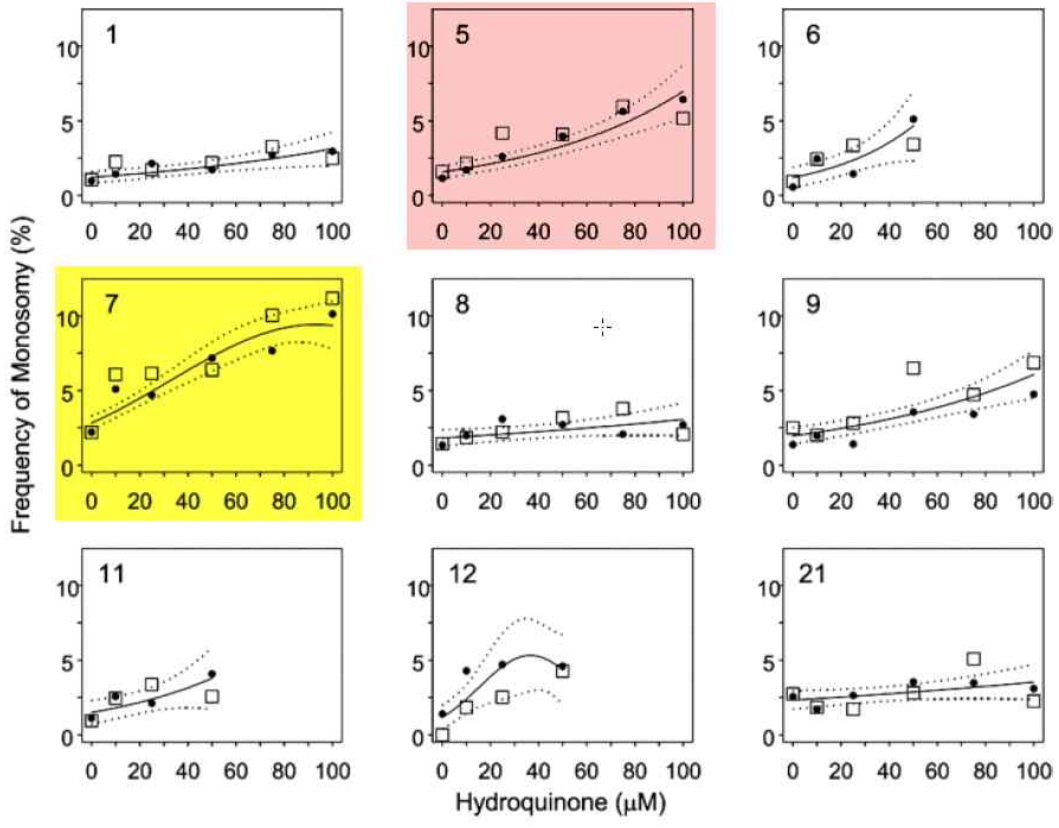


Fig. 1. Frequency of monosomy induced by HQ in chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21. Mean values for monosomy of the nine chromosomes following treatment with 0, 10, 25, 50, 75, and 100 μM HQ in cultured human lymphocytes are presented for each blood donor (as open squares and filled circles). Each donor's blood was cultured in three separate experiments. The graphs represent the best log-linear (or log-quadratic) fit to the actual raw data (solid line) and the 95% pointwise confidence intervals as dashed lines. Data for chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21 are shown sequentially with the chromosome number indicated in the top left corner. Only six chromosomes (1, 5, 7, 8, 9, and 21) were examined at higher doses of HQ (75 and 100 μM) due to its cytotoxicity such that fewer cells were obtained.

(Zhang et al., 2005)

④ Biomarker of Clinical disease prognosis(예후)

○ 논문: Cytogenetic analysis and clinical significance of chromosome 7 aberrations in acute leukemia (Brozek et al., 2003): Leukemia 환자 중 chromosome 7 monosomy를 가지면 치료 후 CR이 낮음

AML: Acute myelogenous leukemia

Table 4. Frequency of complete remission (CR) in the groups of AML patients: with a normal karyotype and with chromosome 7 aberrations in bone marrow cells

Karyotype	Number of patients		
	CR+(%)	CR–(%)	total
Normal karyotype	16 (64)	9(36)	25
Chromosome 7 aberrations	3 (21)	11(79)	14
Total	19 (49)	20(51)	39

CR+ = complete remission achieved.
CR– = not achieved

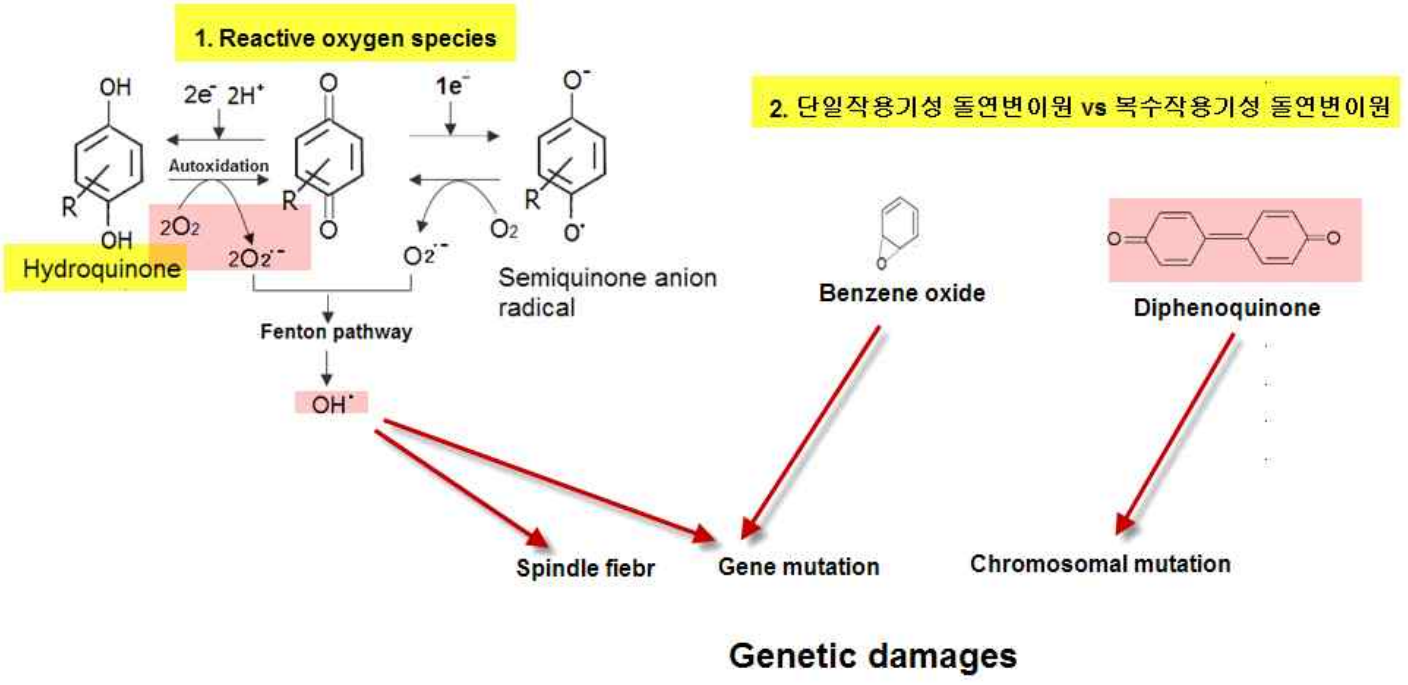
○ The origin model of molecular epidemiology에서 가장 확실한 Biomarker

4. Benzene과 leukemia의 molecular epidemiology에서의 clinical effect에 대한 독성학적 해석

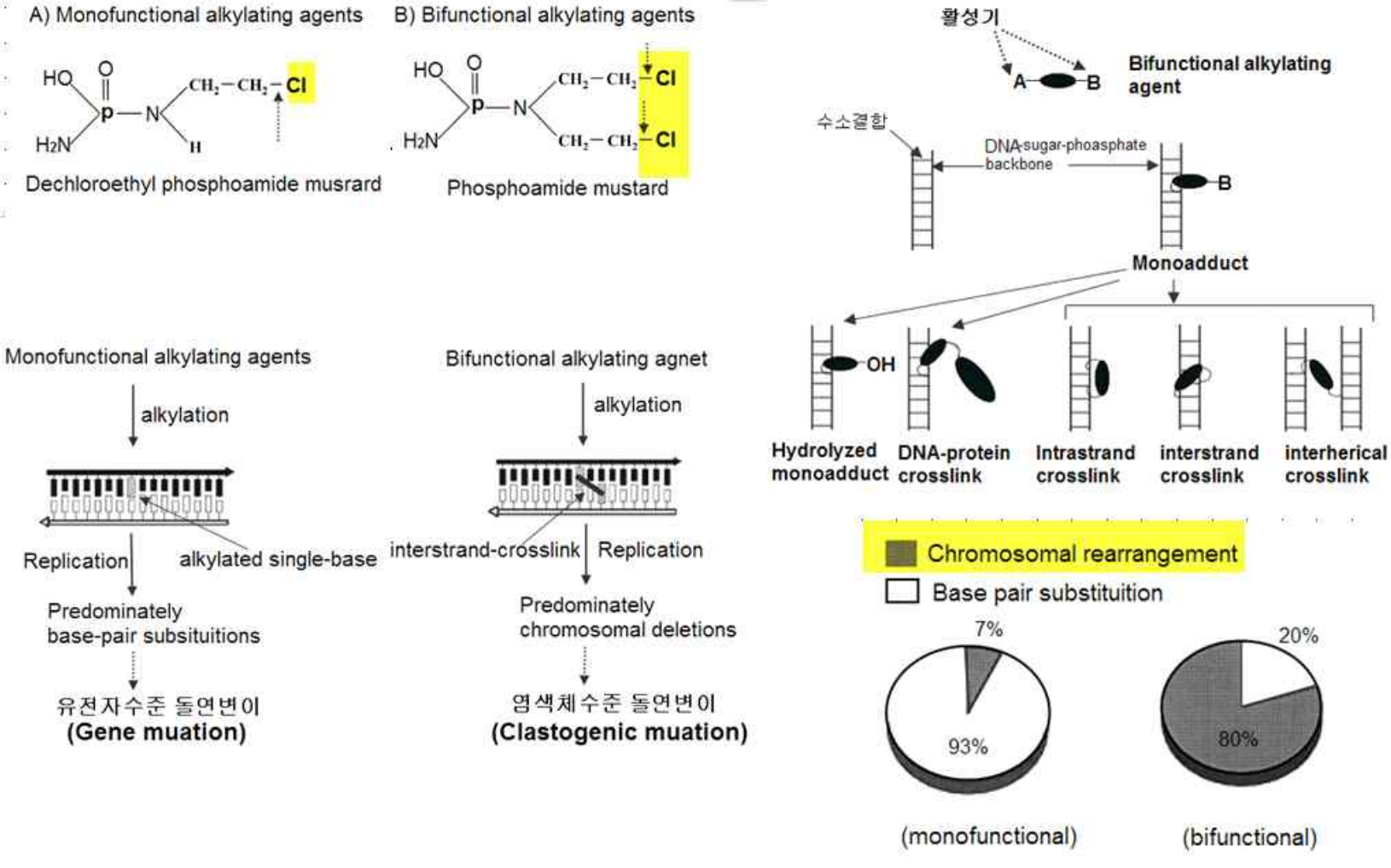
- Benzene produces genomic instability by causing recombination, double strand breaks and mitotic spindle disruption, which leads to formation of translocations, inversions, deletions, and chromosome loss and gain (Smith, 1996)

- 수 많은 mutation 종류 중에 Benzene-specific pattern의 mutation이 없다: 다양한 대사체에 의한 다양한 DNA 손상의 결과로 다양한 mutation

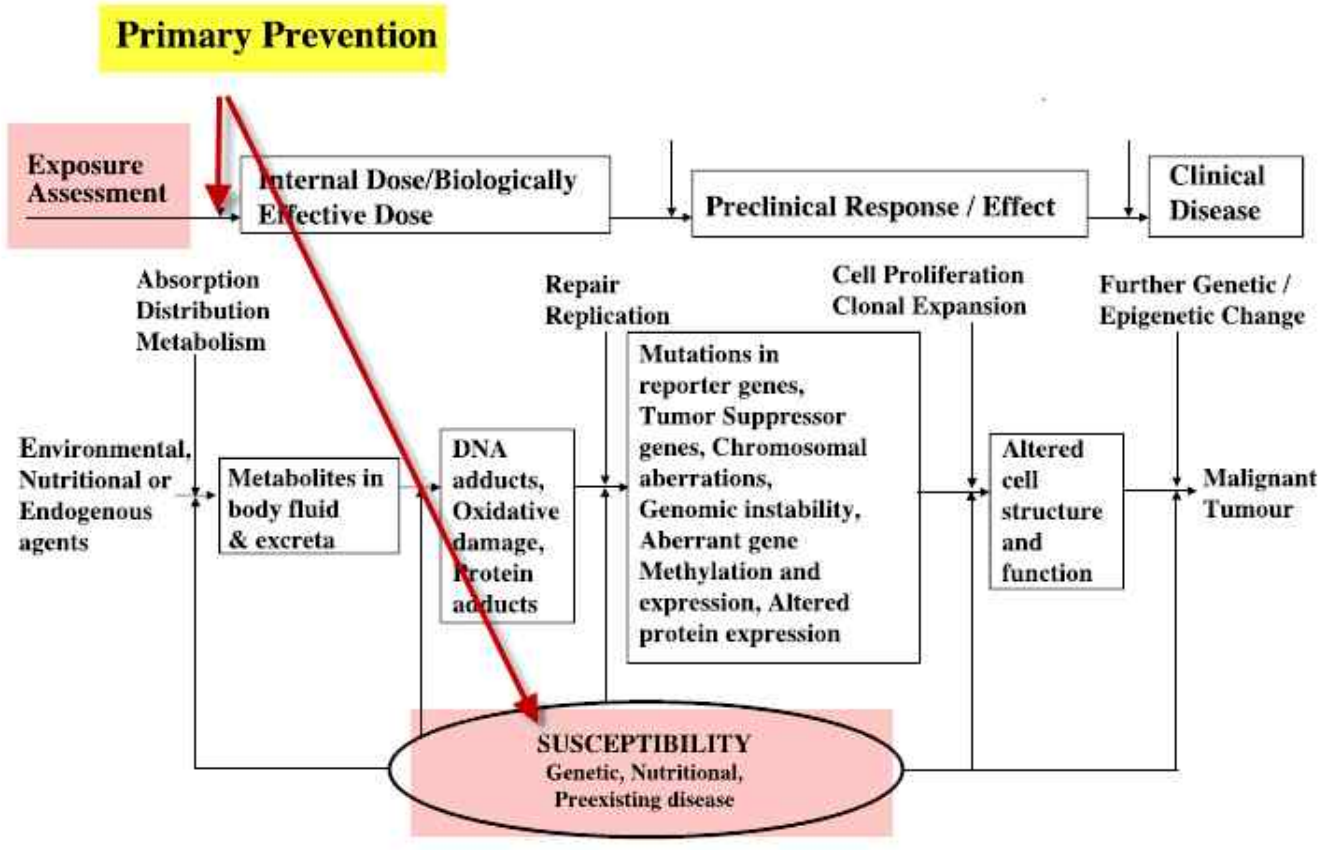
- 원인: Benzene의 단일작용기성 대사체와 복수작용기성 대사체가 혼재



○ Monofunctional agents vs Bifunctional agents



5. Benzene-induced acute myelogenous leukemia 예방을 위한 Biomarker



○ Molecular epidemiology: Biomarker-based epidemiology

1) DNA methylation by benzene exposure – high AML risk group

○ Epigenetic(후생유전) mechanisms of carcinogenesis: mechanisms that do not depend on structural changes in DNA but on functional regulation such as DNA methylation)

○ DNA methylation(메틸화): DNA-methyltransferase (메틸전이효소)에 의해 cytosine 염기에 메틸기(methyl group, CH_3)의 전이반응 – 유전자 발현 억제의 정상적인 기능 CpG island: 유전자의 Promotor 부위에 CpG 염기서열이 밀집한 것

Exon 1 CpG 12634-12767

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11941 ttataagatc ccoctcoctc taaatcctgt ccttctatca cttcatcctt CGctctcctt
12001 taaaatgaga cagttgtcag caggaatcct gCGcaagaac acaccaccct gtttcataga
12061 agatatctca ggtaatgtgc aaacaCGggt ttttaaaCGg agCGcatttt tctcatttgt
12121 taatatcacc acctaaatca tctcttgcct aaaacaagga gtagaaagtg aatgaaggaa
12181 ggaacaggtg atggtcagtg tcctttctac gcctcaaaat ttaagagttt atgtgaaaat
12241 tcataaatat taatctcaat ccaggttaag caaaattttt tgctctcctc tttagaaatt
12301 tctggttgcc aaagttccag aaattgcttc ctcattcctg agcctttcat tttctCGatt
12361 tctccattat gtaaCGggga gctggagctt tgggcCGaat ttccaattaa agatgatttt
12421 ttcagtcaat gagccaCGtc agggagCGat ggcaccCGca ggCGgtatca actgatgcaa
12481 gtttcaagc gaatctcaac tCGttttttc CGgtgactca ttccCGgccc tgcttggcag
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*12601 gggcCGCGCG gcaattggtc ccCGCGcCGa cctcCGccCG CGagCGcCGc CGcttccctt
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*12721 gacagcCGCG gCGcCGCGag cttctcctct cctcaCGacc gaggcaggta aaCGccCGgg
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12961 CGggtgaggc ggcttggctt CGcttttcag gttaggaaag ctccctttac tgCGCGttgg
13021 ggggctgggg gagctggCGg agccaCGtta gggaggtCGg tggCGcCGgg gtgtctcagc
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13261 ccttggcaaa gcctggCGct tcCGCGatgc ccagagggtg cttgggggga tggagagagg
13321 ggCGccCGcc ggggtagttc CGggagcctc ggtgcctccc gcCGcagctg cagCGttcct
13381 ccCGggaggc ggcccagccc ttcatcctCG cCGcctgagc ttctcCGagg ggggctgcac
13441 ccttgCGgcc gttgccacCG cctggagaag CGgccaCGc ggactgaCGg gCGggggCGg
13501 ggcctCGggc ctCGgCGggg gCGgggtcCG gggaggcccc accctctgtt ctccaggggc
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13741 CGccttcaCG tttcattatt aaacaatggg gagaaatcca tgtttactgt ccttttagg
13801 aatttttgc tcttctcttt gaggtggctg taggaaatag attttttttt taacctCGca
13861 attccaccac ggtcacatcc atcctCGcca tCGcagagcc acagctctcc gtttttgttt
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13981 tcatggcCGc gttatttttct tgttctgaga gcatcaCGgt ttaattagca gttcccccata
14041 tgatttgaag tgtttccCGt ttccttaggg aaaactcctg gtagaatagg attaaggatt
14101 tttacaaata taattatcaa aaacatagga acagggaatt ggataaatat gttaaacttc
14161 tggaaaaatc aacaaCGctc ttagatttgt agaagaaagg aaaaaatcac cagtggaaag
14221 gagcaatttt acttacacaa acacagagaa ggtcttacag tgaaaaaaag ctaaccagta
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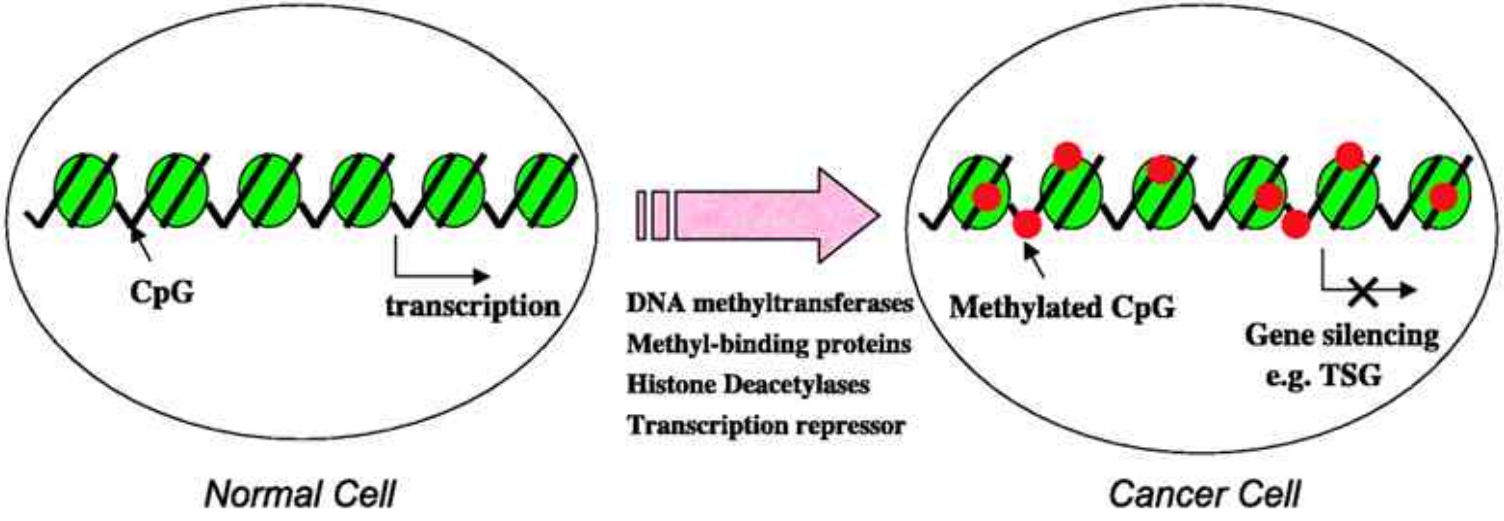
O Cancer cell에서 DNA methylation: 암세포에서는 유전체 전반에서 감소(Global genomic hypomethylation)하지만 정상세포에서 메틸화가 일어나지 않았던 CpG island promotor 부분에 DNA methylation이 증가하며 특정유전자의 발현을 억제한다. (일종의 돌연변이 역할?)



○ A specific pattern: AML(급성골수성 백혈병, Acute myelogenous leukemia)의 gene-specific hypermethylation

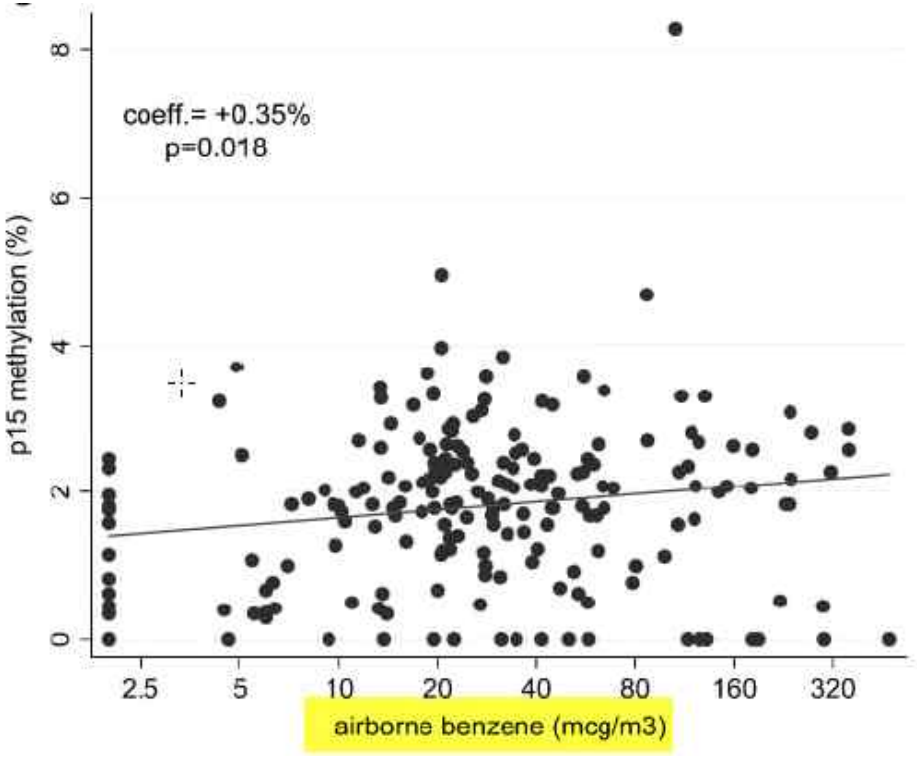
○ In AML, gene-specific hypermethylation shows a specific pattern, including frequent methylation and inactivation of the p15 tumor suppressor gene (Galm et al., 2006)

Table 2 Genes frequently methylated in hematopoietic malignancies.

Tumor subtype	Genes
Non Hodgkin's lymphoma	DAP kinase 1, p57, p16, O^6MGMT, GSTπ, RARβ2, CRBP1
Multiple myeloma	p16, SOCS-1, E-cadherin, p73, DAP kinase 1
Chronic myelogenous leukemia	p15, abl
Acute lymphocytic leukemia	E-cadherin, p16, p15, p73, DAP kinase 1, O^6MGMT
Acute myelogenous leukemia	p15, E-cadherin, SOCS-1, p73, DAP kinase 1, HIC1, RARβ2, CRBP1

(Galm et al., 2006)

○ Hypermethylation in p15 (+0.35%; P = 0.018) were associated with increasing airborne benzene levels.(In article: Changes in DNA Methylation Patterns in Subjects Exposed to Low-Dose Benzene)



(Bollati et al., 2007)

○ Gene-specific analysis showed low p15 methylation in the referent group (mean, 1.2; SD, 0.9) that increased significantly in traffic officers (mean, 2.0; SD, 1.0) and gas station attendants (mean, 2.0; SD, 1.2; P < 0.001).

○ Healthy subjects by low-level exposure to benzene- cancer risk

○ Blood DNA samples- biomarker 가 갖추어야 할 timely, easily 탁월

Table 3. Methylation levels (Cm%) of global and gene-specific methylation markers in the exposed groups (urban traffic officers and gas station attendants) compared with referent subjects (office workers)

	Referents		Benzene-exposed subjects			
	Office workers		Urban traffic officers		Gas station attendants	
	n	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)
LINE-1 (Cm%)*	51	65.7† (5.2)	74	62.3† (6.5)	61	62.2† (6.6)
*Alu*I (Cm%)*	57	27.3 (3.4)	76	27.5 (3.9)	72	26.4 (2.5)
p15 (Cm%)	52	1.2‡ (0.9)	76	2.0‡ (1.0)	74	2.0‡ (1.2)
MAGE-1 (Cm%)	56	93.4 (1.8)	74	93.6 (2.1)	76	93.4 (1.8)

Abbreviations: SD, standard deviation; Cm%, methylated cytosine percentage.
*DNA methylation of *LINE-1* and *Alu*I repeated elements was measured as a surrogate for global DNA methylation (10).
† $P = 0.003$, ANOVA test for differences among exposure groups.
‡ $P < 0.001$, ANOVA test for differences among exposure groups.

(Bollati et al., 2007)

2) Benzene- exposure susceptibility : cytochrome P450의 SNP를 통한 개인차

○ SNP(Single Nucleotide Polymorphism)에 의한 benzene susceptibility

○ 단일염기변이라고 하며 인종이나 개인별 염기의 차이를 말한다. 세포핵 속에 염색체가 갖고 있는 30 억개의 염기 서열 가운데 개인 편차를 나타내는 한 개 또는 수십개의 변이염기를 일컫는다. 500～1000 염기당 1개꼴로 나타나며, 인간지놈에는 약 300만개의 SNP가 존재하고 있다.

○ 인간은 인종이나 민족과 상관없이 유전자가 99.9% 일치하지만 0.1%의 SNP 때문에 키와 피부색이 달라지게 된다. 한국인이 서양인에 비해 위암과 간암이 잘 걸리는 것도 이런 차이에서 생기는 것으로 추정되고 있다.

예) Cytochrome P4502E1

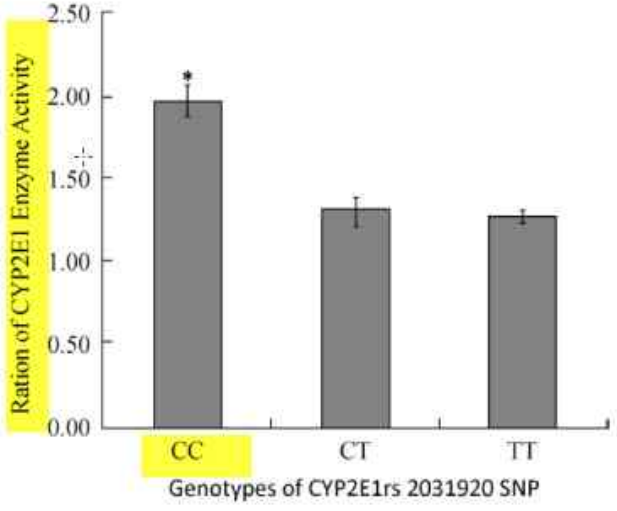
Table 1. Information of SNP in Locus Region in Asian Population from dbSNP NCBI

RefSNPID	Position in Gene	Function Class	Ref SNP Alleles	Ancestral Allele	Alleles (Ancestral)	Alleles (Mutant)
Rs2070673	-298	Locus region	A/T	A	0.440-0.500	0.500-0.560
Rs2031921	-992	Locus region	C/T	T	0.812	0.188
Rs2031920	-1020	Locus region	C/T	C	0.711-0.812	0.167-0.289
Rs3813866	-1531	Locus region	A/T	T	0.717	0.283

(Zhang et al., 2010)

○ Cytochrome P5402E1(CYP2E1)의 SNP(single nucleotide polymorphism, 단일염기 변이)이 활성이 높은 사람이 benzene toxicity에 susceptibility가 높음 :

○ 중국인 혈액세포에서 유전자 손상 정량분석법(Comet assay)의 Comet rate이 CYP2E1 SNP rs2031920에서 높음



Phenol 투여 후 lymphocyte에서 단백질 활성

Table 4. DNA Damage by Phenol in Cells with CYP2E1 rs2031920 SNP

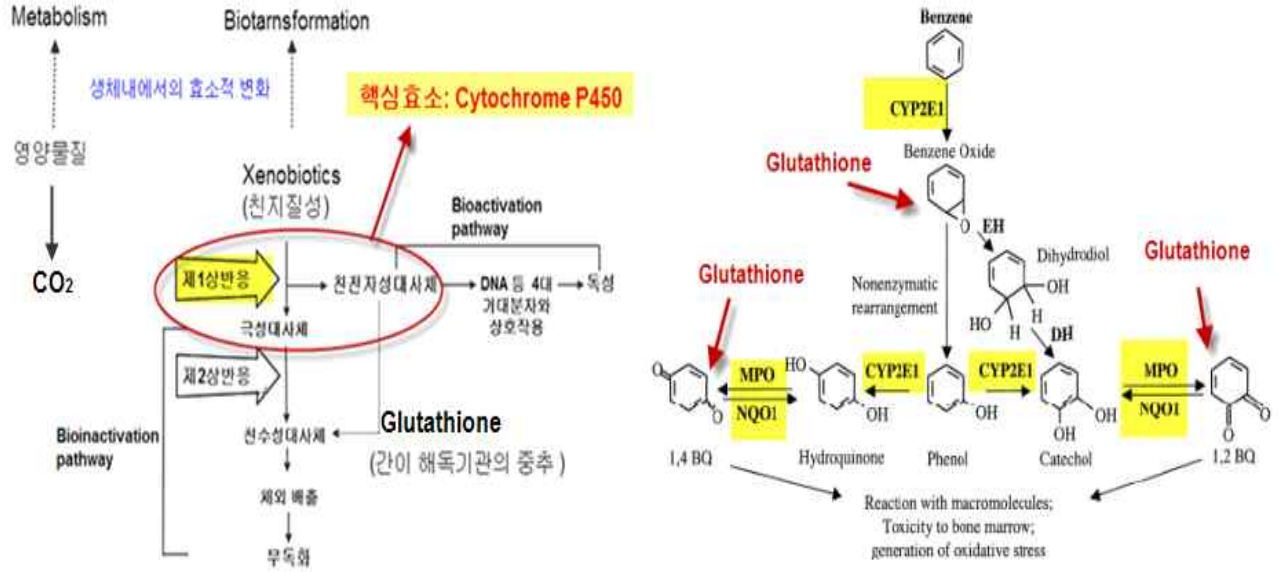
Genotype	Groups	Comet rate (%)	Ratio of comet rate
rs2031920 CC	Control	3.22±5.09	7.26±1.04*
	0.01%	22.28±4.02*	
rs2031920 CT/TT	Control	3.56±1.17	4.39±1.38
	0.01%	13.17±3.19*	
rs2070673 TT	Control	3.33±0.86	5.23±1.53
	0.01%	16.50±2.16*	
rs2070673 AA/AT	Control	3.44±1.06	4.23±1.58
	0.01%	13.56±2.93*	

(ZHANG et al., 2010)

6. Benzene과 leukemia의 molecular epidemiology에서의 Primary prevention 에 대한 독성학적 해석

1) DNA methylation by benzene exposure – high AML risk group
2) Benzene- exposure susceptibility : cytochrome P450 SNP를 통한 개인차

○ Cytochrome P5402E1 (CYP2E1)이 활성이 높은 사람이 benzene toxicity에 susceptibility가 높음



(Park, 2010)

○ 동물과 사람에 있어서 발암물질에 대한 susceptibility의 차이에서 가장 큰 이유: Cytochrome P5402E1(CYP2E1) 종류의 차이에 기인

Complement of rat liver cytochrome P450 (CYP) enzymes in uninduced and Aroclor-1254 induced states

Enzyme	Cytochrome P450 (nmol/mg microsomal protein)	
	Untreated	Aroclor treated
CYP1A1	0.04	1.45
CYP1A2	<0.03	1.23
CYP2B1	0.03	1.29
CYP2B2	0.07	1.46
CYP2C6	0.36	0.36
CYP2C11	1.20	0.27
CYP2D1	0.15	0.15
CYP3A	0.39	0.77

유전독성에서의 S9 Fraction: Metabolic activation

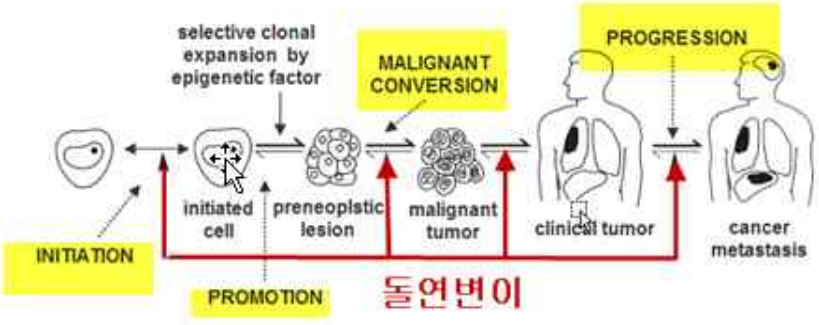
Table 1. Classification of Human P450s Based on Major Substrate Class

sterols	xenobiotics	fatty acids	eicosanoids	vitamins	unknown
1B1	1A1	2J2	4F2	2R1	2A7
7A1	1A2	4A11	4F3	24A1	2S1
7B1	2A6	4B1	4F8	26A1	2U1
8B1	2A13	4F12	5A1	26B1	2W1
11A1	2B6		8A1	26C1	3A43
11B1	2C8			27B1	4A22
11B2	2C9				4F11
17A1	2C18				4F22
19A1	2C19				4V2
21A2	2D6				4 × 1
27A1	2E1				4Z1
39A1	2F1				20A1
46A1	3A4				27C1
51A1	3A5				
	3A7				

5가지 P450이 95%에 관여

7. Benzene-induced AML에서 DNA methylation의 독성학적 의미

○ **Chemical carcinogenesis(화학적 발암화)의 Multi-stage theory (다단계이론):**세포의 발암은 정상세포가 다양한 종양전구(pre-neoplastic) 또는 종양성(neoplastic) 표현형 및 유전형을 발현하면서 여러 번의 돌연변이 과정의 다단계 즉 개시-촉진-악성전환-진행 에서 돌연변이를 통해 암세포로 전환되는 매우 희귀한 과정이다.



발암화의 다단계와 단계별 특성과 돌연변이: 대부분의 발암화는 개시(initiation), 촉진(promotion), 악성전환(malignant tumor) 그리고 진행(progression) 단계 구성되는데 각 단계는 추가적인 돌연변이에 의해 진전된다. (참고: Weston)

(Park, 2010)

7. Benzene-induced AML에서 DNA methylation의 독성학적 의미

○ 발암의 multi-stage와 암세포전환을 위한 돌연변이 유전자의 수

- 지금까지 발암을 위해 요구되는 돌연변이의 최소 유전자수는 대장암(colon cancer)이의 4개이다.
- 결장암과 관련하여 돌연변이의 주요 4개 유전자는 APC(adenomatous polyposis coli), K-ras, DCC(deleted in colon cancer), 그리고 p53 등이다.

○ 암의 원인을 모른다는 것은 어떤 암에 어떠한 유전자의 돌연변이가 발생하며 그 수는 얼마나 되는가에 대한 명확하게 설명할 수 없기 때문이다

○ 암을 유발하는 유전자 돌연변이 존재: 120여 종류의 인체세포에 120 여종의 암종이 존재. 사람의 전체 유전자 22,500개 중 약 **2% 유전자의 돌연변이가 발암**과 관련이 있다. 이중 **생식세포에서는 암과 관련된 유전자는 약 70개, 체세포에서는 약 342개의 유전자가 관련이 있는 것을 추정되고 있다.**

○ 2% 유전자: 3 종류인 Proto-oncogene(발암전구유전자), tumor-suppressor gene(발암억제유전자)와 DNA mismatch-repair gene(DNA 수선유전자)

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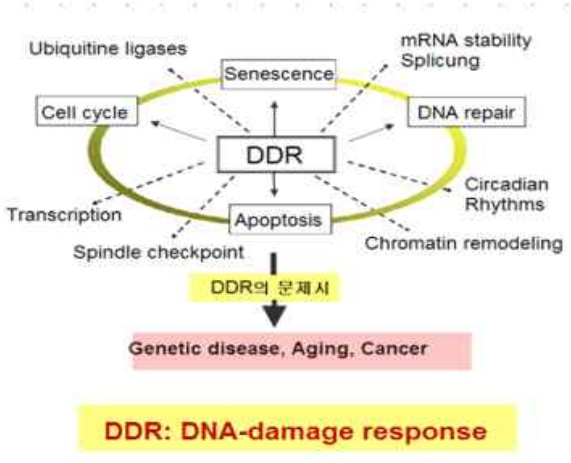
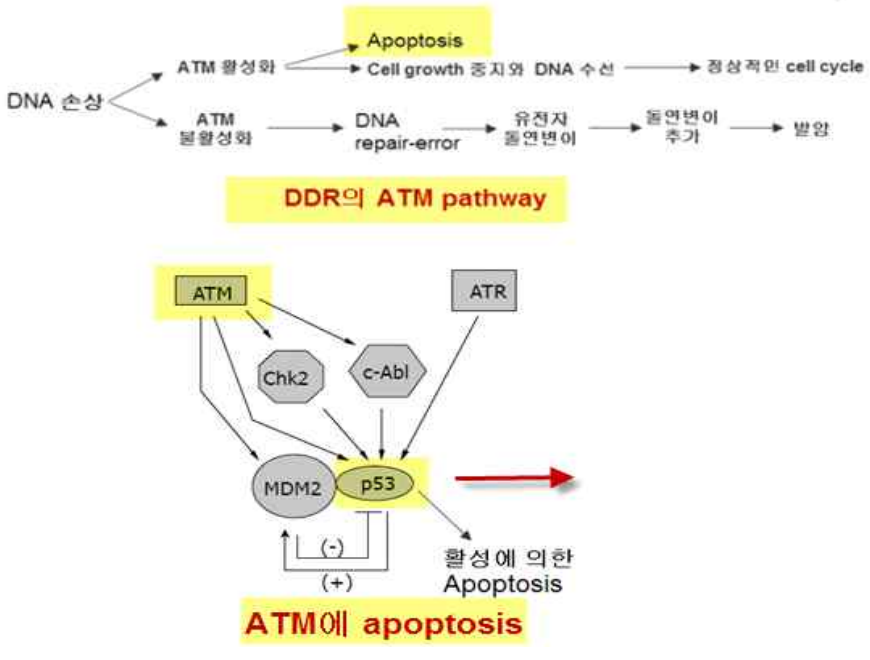
7. Benzene-induced AML에서 DNA methylation의 독성학적 의미

○ 2% 유전자인 3 종류 Proto-oncogene(발암전구유전자), tumor-suppressor gene(발암억제유전자)와 DNA mismatch-repair gene(DNA 수선유전자) 의 돌연변이에 의해 발암 -> 기존의 이들 유전자의 기능을 억제.

○ p53(유전자 TP53) 돌연변이는 모든 암의 50%에서 발생에 주요 원인





(Park, 2010)

7. Benzene-induced AML에서 DNA methylation의 독성학적 의미

○ AML의 환자에서 나타나는 돌연변이 유전자는 약 11개: 대부분Proto-oncogene, tumor-suppressor gene와 DNA mismatch-repair gene 추정

Table 2
Frequency of molecular mutations in AML *de novo*.

Mutated gene	AML *de novo*
FLT3 (ITD)	35%
FLT3 (TKD)	9%
NRAS	10–15%
KIT^{D816}	~5%
MLL (ITD)	3%
RUNX1	10–15%
TP53	10%
PTPN11	~2%
NPM1	35–50%
CEBPA	6–15%
JAK2^{V617F}	2–5%

Table 2 Genes frequently methylated in hematopoietic malignancies.

Tumor subtype	Genes
Non Hodgkin's lymphoma	DAP kinase 1, p57, p16, O^6MGMT, GSTπ, RARβ2, CRBP1
Multiple myeloma	p16, SOCS-1, E-cadherin, p73, DAP kinase 1
Chronic myelogenous leukemia	p15, abl
Acute lymphocytic leukemia	E-cadherin, p16, p15, p73, DAP kinase 1, O^6MGMT
Acute myelogenous leukemia	p15, E-cadherin, SOCS-1, p73, DAP kinase 1, HIC1, RARβ2, CRBP1

(Galm et al., 2006)

○ AML에서의 DNA methylation 유전자: AML에서도 DNA Methylation의 대부분이 Proto-oncogene, tumor-suppressor gene와 DNA mismatch-repair gene에서 발생

Table 2 Genes frequently methylated in hematopoietic malignancies.

Tumor subtype	Genes
Non Hodgkin's lymphoma	DAP kinase 1, p57, p16, O^6MGMT, GSTπ, RARβ2, CRBP1
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Acute myelogenous leukemia	p15, E-cadherin, SOCS-1, p73, DAP kinase 1, HIC1, RARβ2, CRBP1

Table 1 Pathways disrupted by aberrant CpG island methylation associated with gene silencing in human cancer.

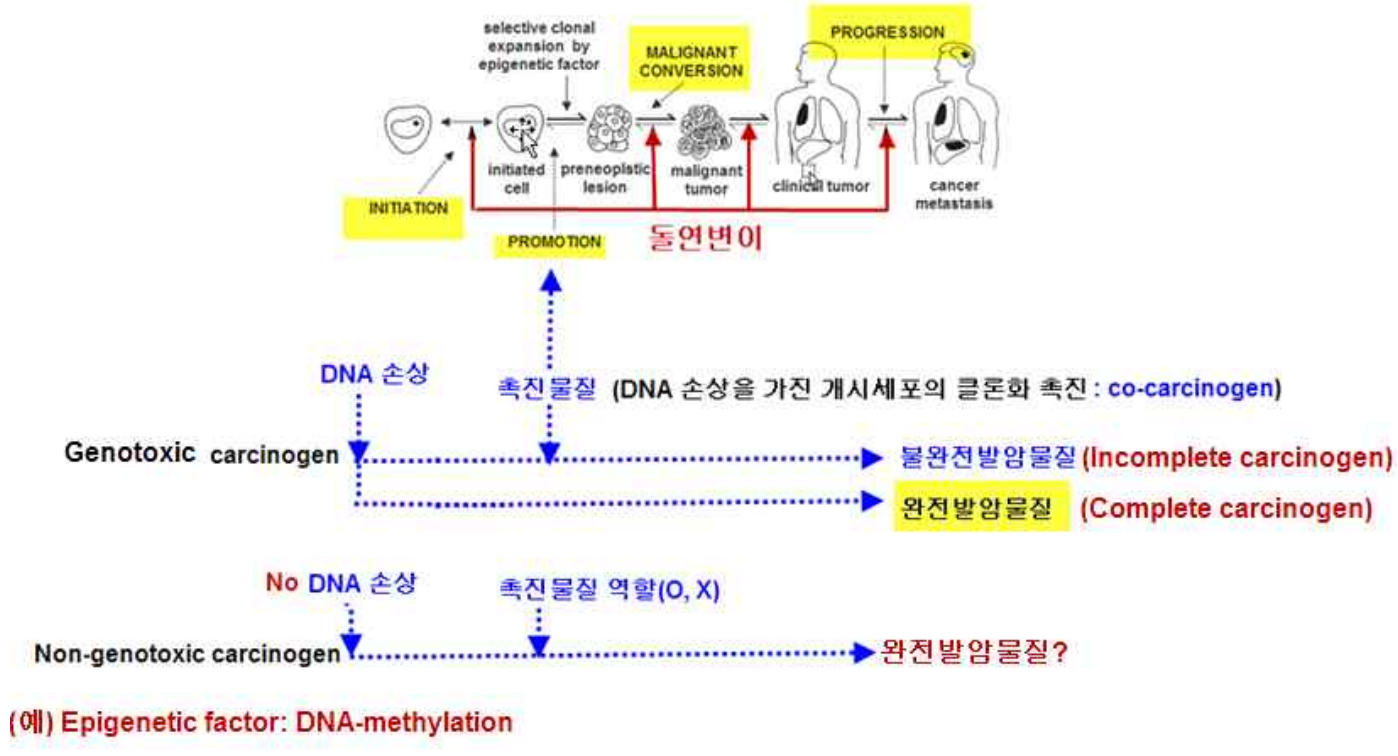
Pathway	Genes
Cell cycle control	Rb, p14, p15, p16, p73
Cell invasion and adhesion	E-cadherin, APC, TIMP-3, VHL
Regulation of apoptosis	DAP kinase 1, caspase 8, TMS-1
DNA damage repair	O^6MGMT, hMLH1, BRCA1, GSTπ
Growth-factor response	RARβ2, CRBP1, SOCS-1, SOCS-3, ER

(Galm et al., 2006)

7. Benzene-induced AML에서 DNA methylation의 독성학적 의미

○ Benzene-induced methylation vs Benezne-induced mutation: 공통적으로 Proto-oncogene, tumor-suppressor gene와 DNA mismatch-repair gene에서 발생

○ DNA-methylation은 완전발암물질의 분류되는 위상?



(Park, 2010)

결론: 분자역학의 독성학적 이해

○ 본 연구에서는 **Benzene**과 **Leukemia**를 The origin model of "Molecular Epidemiology"에 대입하여 **Biomarker requirement에 얼마나 부합하는가를 확인과 더불어 독성학 의미를 살펴보았다. (Biomarker requirements :** Timely , Easily(non-invasive), Specificity and Sensitivity).

○ Clinical prevention의 biomarker는 Benzene-specific mutation pattern이 없는 것으로 추정된다.

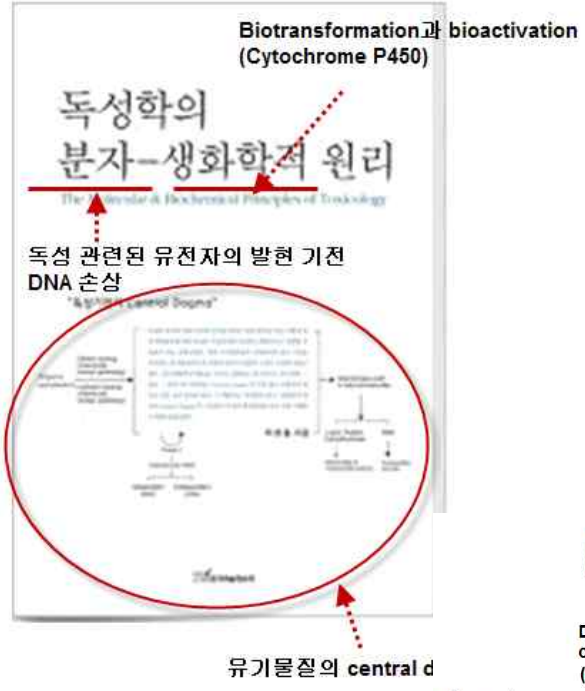
○ AML의 Clinical disease 측면에서 Chromosome 7의 monosomy는 complete remission에 대한 좋은 biomarker 가능성

○ Primary intervention의 biomarker 측면에서 p15 gene - specific hypermethylation이 benzene-induced AML의 risk biomarker로 활용가능성

○ **Benzene**과 **Leukemia**의 분자역학을 통한 독성학적 이해:

1. 독성대사체의 생성 종류는 Biomarker의 Chemical-specific mutation pattern에 큰 영향을 주는 것을 확인
2. 독성물질의 Biomarker는 제한적이지만 필드 활용 가능성은 충분히 있다는 점
3. Chemical carcinogenesis에서 multi-stage theory에서 epigenetic factor (예: DNA methylation)의 중요성이 새롭게 반영되어야 할 필요성

<독성학의 분자-생화학적 원리>



<Central dogma>