CHAPTER II

LITERATURE REVIEW

Leukemias

Cancer in children under 15 years old is rare accounting for less than 1% of all malignancies diagnosed each year. (Coleman *et al.,* 1999; Draper, 1995). Childhood cancers tend to differ from those diagnosed in adults in terms of their site of occurrence, histological, appearance, and clinical behavior ---growing rapidly, being aggressively invasive, and being more responsive to chemotherapy (Miller, 1995). Leukemia are the most common cancer to effect children, accounting for between 25% and 35% of all childhood cancers (Parkin *et al.*, 1988). A larger number of haematological malignancies are seen in boys compared to girls, the greatest differences being observed for lymphomas where between two-thirds and three-quarters are male (Parkin *et al.*, 1988; UK Childhood Cancer Study Investigators, 2000).

World Health Organization (WHO) classification of leukemias (The American Journal of Surgical Pathology, 1997, 21(1): 114-121)

Acute lymphoblastic leukemia (ALL)

Acute lymphoblastic leukemia (L1/L2)

Precursor B cell

Precursor T cell

Acute lymphoblastic leukemia, B-cell (L3)
(equivalent to Burkitt lymphoma in leukemic phase to be discussed with Burkitt lymphoma)

Lymphoblastic lymphoma/leukemia

Precursor B-cell

Precursor T-cell

Acute leukemia, biphenotypic

Acute myeloid leukemia (AML)

Acute myeloid leukemia, minimally differentiated (MO)

Acute myeloid leukemia without maturation (M1)

Acute myeloid leukemia with maturation (M2)

Acute myeloid leukemia with maturation with t(8;21)

Acute promyelocytic leukemia (M3)

Hypergranular type

Microgranular type

Acute myelomonocytic leukemia (M4)

Acute myelomonocytic leukemia with increased marrow eosinophils (M4EO)

Acute Monocytic Leukemia (M5)

Acute monoblastic leukemia (M5a)

Acute monocytic leukemia with maturation (M5b)

Erythroleukemia

Erythroid /myeloid) (M6a)

Pure erythroid malignancy (M6b)

Acute megakaryoblastic leukemia (M7)

Acute megakaryoblastic leukemia associated with t(I;22)

Acute basophilic leukemia

Acute myelofibrosis (acute myelodysplasia with myelofibrosis)|

Acute leukemia and transient myeloproliferative disorder in Down's Syndrome

Hypocellular acute myeloid leukemia

Myeloid sarcoma

The biological heterogeneity of childhood leukemia is well documented (Biondi *et al.*, 2000; Kersey, 1997), with the major morphological types being acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML). ALL is the predominant leukemia seen in childhood, with AML accounting for around 15 – 17% of case in white populations and slightly higher proportion in Asia and black populations of North America (Parkin *et al.*, 1988). Chronic forms of leukemia in children are very rare in all populations and seldom exceed 4% of all leukemia diagnoses.

Subtypes of AML and ALL are frequently characterized by genetic alterations, including point mutations and deletions, as well as chromosomal translocations, including chimeric or fusion genes and changes in chromosome number (hyperdiploidy or hypodiploidy) (Look, 1997; Raimondi, 1999). Although over 200 genes have been found to be involved in chromosomal translocations, many of them are rare and only certain genes have been associated with childhood leukemia (Table 2) (Greaves, 2003). These include *MLL, TEL,* and *AML1,* all of which can fuse with over 15 other genes and in the case of *TEL* and *AML1* with each other (GOLUB *et al.*, 1995).

Table 2 The major biological subtypes of childhood leukemia and associated chromosomal changes

Subtype	Cell type involved	Chromosome abnormality	Molecular lesion	Frequency (%)	Functional product
ALL	B-cell Progenitor-monocytic[a] (infants)	11q23 translocations	*MLL-AF4*, *MLL-ENL*, and other fusions	~85% of infant ALL ~5 %of total ALL	Modified transcription factor[b]
	B-cell precursor	Hyperdiploidy	Increased gene dosage	~35 %of B- cell precursor	Unknown
		t(12;21) (p13;q22)	*TEL- AML1* fusion	~20 %of B- cell precursor	chimeric transcription factor[c]
		t(1;19) (q23;p13)	*E2A- PBX1* fusion	~5 %of B-cell precursor	chimeric transcription factor
		t(9;22) (q34;q11)	*BCR- ABL* fusion	~5 %of B-cell precursor	Activated kinase
	T-cell precursor	1q deletion;t(1;14) (p32;q11)	*SIL-SCL* fusion	~25 %of T-cell precursor	Dysregulated transcription factor

Table 2 The major biological subtypes of childhood leukemia and associated chromosomal changes. (continued)

Subtype	Cell type involved	Chromosome abnormality	Molecular lesion	Frequency (%)	Functional product
AML	Infant	11q23 translocations	*MLL-AF6, -AF9, -AF10,* and other fusions	~50 %of infant AML	Modified transcription factor[b]
		t(8;21)(q22;q22)	*AML-ETO* fusion	~15 %of total AML	chimeric transcription factor[c]

Adapted from Greaves and Wiemels (2003).

[a]This subtype has phenotypic features of both early B-lineage progenitors and monocytes.

[b]MLL may modify chromatin structure and control the expression of key developemental genes (such as *HOX*).

[c]AML-1, which is an important positive transcriptional factor, may be switched to a transcriptional repressor in these fusions (Tenen, 2003; Speck and Gilliland, 2002).

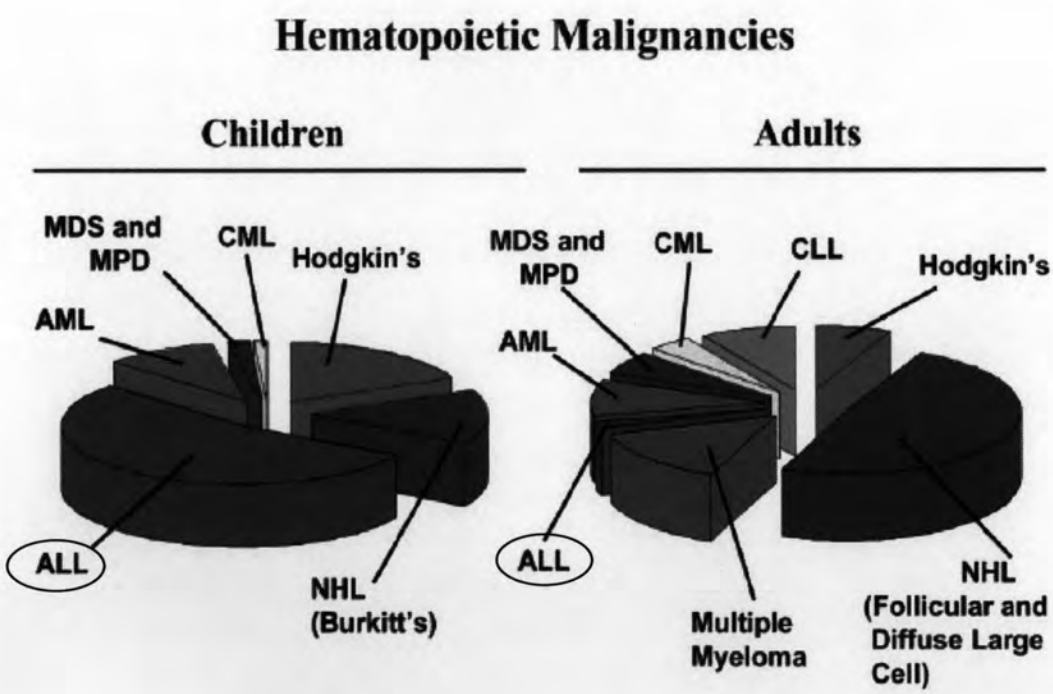
The incidence of leukemia rises rapidly after birth peaking at around 3 years of age before declining and then rising steadily again throughout life (Linet, 1991). The different subtypes of ALL tend to segregate by age, which may account for the marked prognostic differences among infants, children, and adults (Greaves, 1999). In infants less than 12 months old, a distinet type of ALL with pro-B phenotype and chromosome rearrangements in the *MLL* gene has been identified. The marked peak in incidence between 2 and 5 years of age, associated with common B-cell ALL, has

resulted in many etiological hypotheses, most notably those involving exposure to infections (Greaves, 1993; Kinlen, 1995; Law *et al.*, 2003).

Acute lymphoblastic leukemia (ALL)

Leukemia, the number one disease killer of children less than 14 years of age in the United States, represents around 31% of all cancer cases among children in this age group. The incidence of leukemia is 10-fold lower in children than adults, with approximately 3250 children and adolescents younger than 20 years of age diagnosed each year in the US, of which 2400 are acute lymphoblastic leukemia (ALL).

Figure 1 Frequency of the major subtypes of hematopoietic malignancies in pediatric and adult patients.



Leukemia is a heterogeneous disease, characterized by the dysregulated proliferation of blood precursor cells of myeloid or lymphoid origin. It can be classified as acute (low level of differentiation) or chronic (high level of differentiation) and can be further classified by cytogenetic subtype. For example, t(12;21) which generates the TEL- AML1 fusion gene occurs in 25% of patients with common ALL (cALL), a subtype of ALL with peak incidence between ages 2 and 5 (Romana *et al.*, 1995). Translocation t(1;19) and high hyperdiploidy (>50 chromosomes) are also common in childhood ALL (Harrison, 2001), while various specific chromosome rearrangements, including t(8;21), t(15;17), and inv(16), are found in acute myeloid leukemia (AML) (Hall, 2001).

The cause of ALL remain unclear. It is thought to represent the culmination of evolution of an abnormal clone through successive genetic changes. ALL may be considered a complex disease in which the individual's risk of cancer represents a cumulative effect of a series of low-penetrance genes combined with the external factors. A variety of environmental factors were identified to be associated with the risk of ALL. For example, cigarette smoke exposure, dietary effects, etc (Jang-Ming Lee *et al.*, 2001). As with most other cancers, the mechanism by which leukemia arises is likely to involve gene-environment interactions---the environmental exposures being derived from both endogenous and exogenous sources. Accordingly, it is important to identify exposures that cause DNA damage and induce chromosome breaks which are inadequately repaired, ultimately leading to disease initiation and progression.

Figure 2 Bone marrow aspirate from a child with T-cell acute lymphoblastic leukemia. The marrow is replaced with lymphoblasts of various sizes. No myeloid or erythroid precursors are seen. Megakaryocytes are absent.

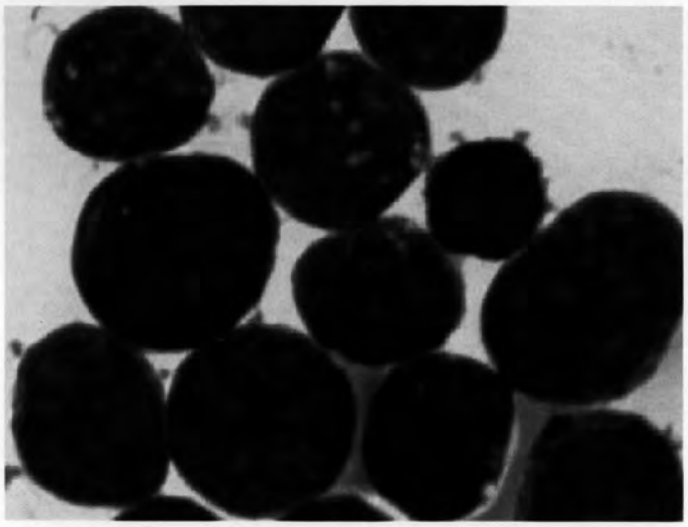
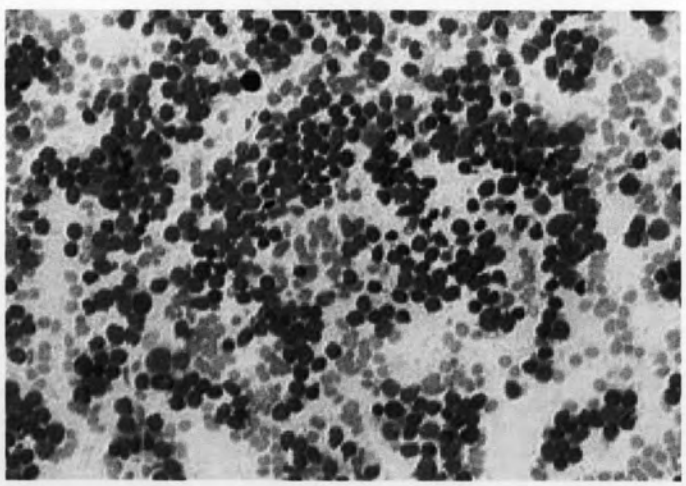


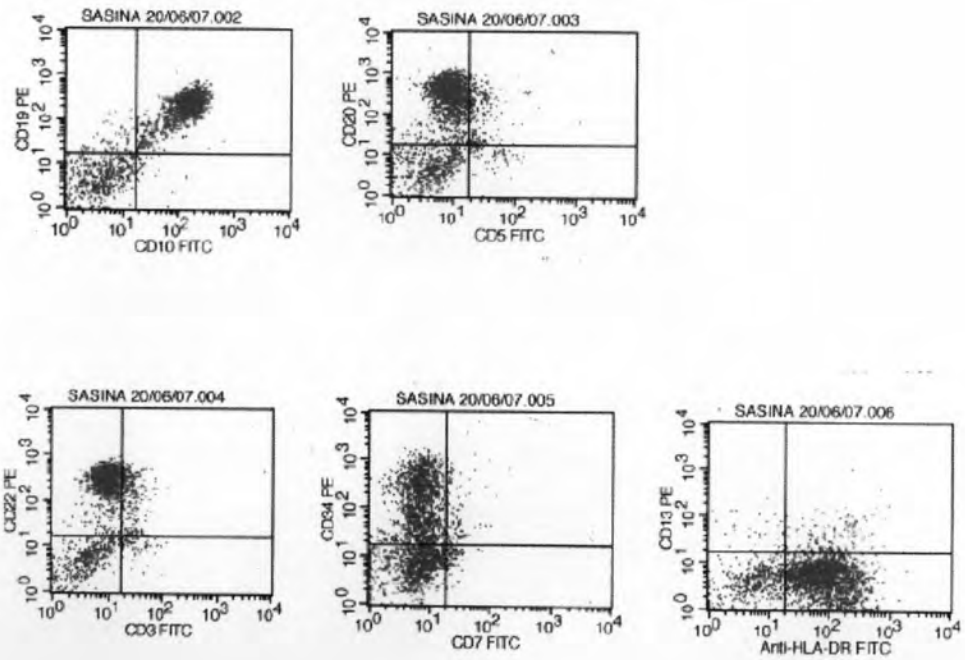
Figure 3 Bone marrow aspirate from a child with B-cell acute lymphoblastic leukemia. The lymphoblasts are large and have basophilic cytoplasm with prominent vacuoles.



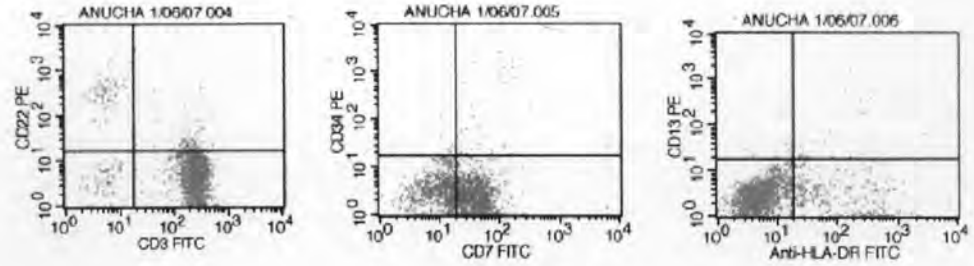
Identifying subtype of the leukemia by immunophenotyping, approximately 80% of childhood ALL involve lymphoblasts with phenotypes that correspond to those of B-cell progenitors. These cases can be identified by their cell-surface expression of 2 or more B-lineage–associated antigens, CD19, CD20, CD24, CD22, CD21, or CD79. Only CD79 is specific for B-lineage ALL (Giordano *et al.*, 2003). T-cell ALL is identified by the expression of T-cell–associated surface antigens, of which cytoplasmic CD3 is specific and HLA-DR negative (Figure 4).

Figure 4 Immunophenotyping by flow cytometry : Acute lymphoblastic leukemia (ALL) : A; B-cell ALL, B; T-cell ALL

A



B



Acute lymphoblastic leukemia in Childhood of Thailand

The previous study of Thai Pediatric Oncology Group (ThaiPOG) was initially review in 2003. ThaiPOG has successfully built a collaborative network for registry new cases of large clinical centers that serve the pediatric population in Thailand. The incidence of childhood cancers in Thailand in 2003 was determined from the cancer registrations collected from 18 pediatric cancer centers around the country. The incidence was compared with similar analyses done at cancer registries in Asia, Europe and the USA. The incidence in Thailand was lower than some Asian and Western countries.

Between January - December 2003, 999 newly diagnosed cases of childhood cancer were registered. Of these patients, 566 (56.7%) were boys and 433 (43.3%) were girls, male: female ratio = 1.3 : 1. The mean and median ages were 6.5 (SD = 0.13) and 5.0 (0-14) years, respectively. Classification the cancer type by International Classification of Childhood Cancer (ICCC), acute leukemia is the most common malignancy in children (53% of all cases). 73.5% were acute lymphoblastic leukemia (ALL) and 22.5% were acute non-lymphoblastic leukemia (ANLL). The incidence of leukemia was 42.6 : 1,000,000 person-year, 43.7 for males and 41.5 for females (Europe and North America, where rates generally ranged from 4.8 : 1,000,000 to 7.4 :1,000,000 for males and 3.2 to 4.6 for females), 31.8 for ALL and 9.2 for ANLL, ALL: ANLL = 3.3:1. Leukemia is the most in 0-4 years old patients (www.ThaiPOG.org).

Acute myeloid leukemia (AML)

Acute myeloid leukemia (AML) or acute non-lymphoblastic leukemia (ANLL) accounts for approximately 20% of acute leukemia seen in children (Weinstein, 1997). In contrast to ALL, the annual incidence of AML is constant from birth to 10 years of age and peaks slightly in adolescence. The ratio of ALL to AML in children under 15 years old is 4:1, however, a neonate with leukemia is more likely to have AML than ALL (Weinstein, 1997). AML is distributed equally among ethnic groups and men and women appear to be affected equally (Arceci, 2002). The principle defect in AML appears to be an arrest in the differentiation pathway of myeloid progenitors or precursors rather than abnormal growth kinetics. The molecular mechanism that leads to a block in differentiation mostly is unknown except in the case of acute promyelocytic leukemia, which results from the fusion of the PML and RAR α genes caused by the t (15; 17) (Weinstein, 1997). Pharmacologic doses of all-trans-retinoic acid are used to overcome the block in differentiation for patients with acute promyelocytic leukemia.

AML has an event-free survival of 25-50% (Creutzig U, 1995; Ravindranath Y, 1996; Stevens RF, 1998), which is markedly lower than the reported evet-free survival of 80% for pre-cursor B-acute lymphoblastic leukemia (ALL) patients (Pui CH, Evoms WE, 1998). Although aggressive induction therapy results in remission rates of up to 90% (Stevens RF, 1998; Woods WG, 1998), many children with AML relapse. At relapse, the prognosis for patients with AML is generally poor, which might be associated with the presence of primary or secondary cellular drug resistance (Klumper E, 1995; Kaspers GJL, 1999).

Figure 5 Bone marrow aspirate from a child with acute myeloid leukemia (AML) or acute non-lymphoblastic leukemia (ANLL).

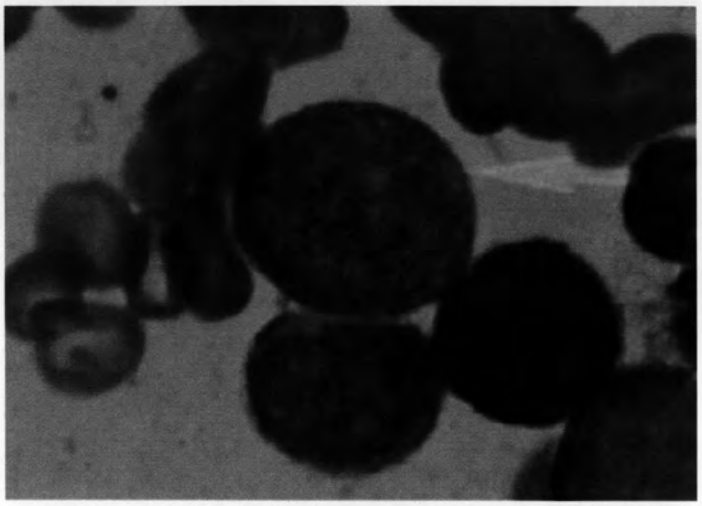
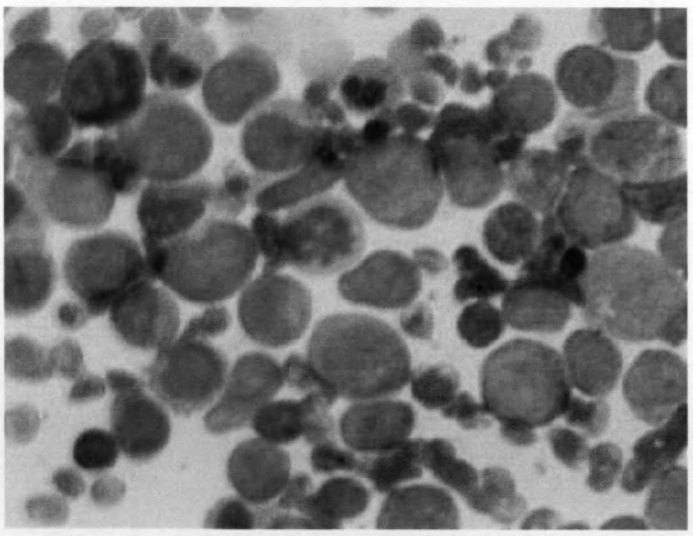
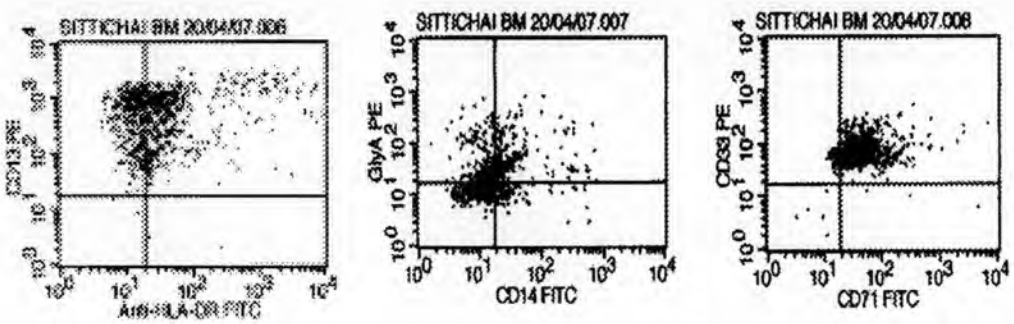


Figure 6 Bone marrow aspirate from a child with AML. The marrow is replaced with myelooblasts. No lymphoid are seen. Megakaryocytes are absent.



AML involve myeloblasts is classified by use of the cell lineage and maturation characteristics. This is the contrast of ALL, which generally show the homogeneous marker expression. The immunophenotypic characteristic of AML, are positive for the panmyeloid marker CD14 and CD71 (figure 7) (Pui *et al.*, 2006).

Figure 7 Immunophenotyping by flow cytometry: Acute myelocytic leukemia (AML)



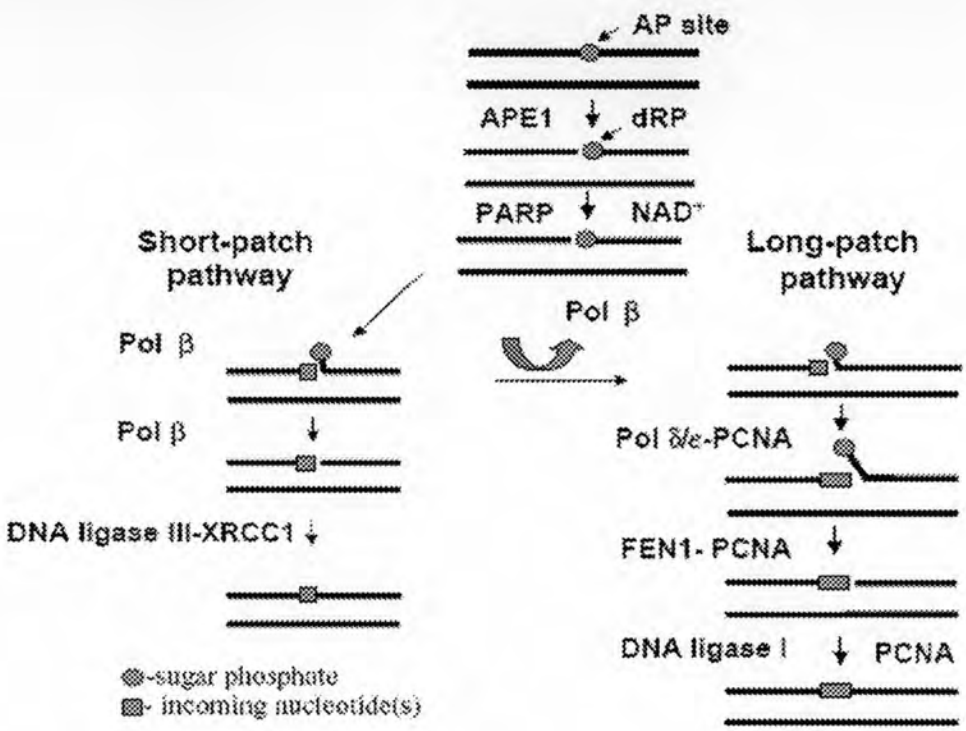
DNA repair

Exposure to endogenous and exogenous carcinogens, and genotoxic compounds can cause damage to DNA. DNA repair process monitors and repairs these DNA alterations using the complex mechanisms. The repair systems include base excision repair (BER), nucleotide excision repair (NER), mismatch repair, and double strand break repair depending on the type of the damaged DNA (Mohrenweiser HW, 1998).

The BER process replaces a single damaged nucleotide with a normal residue. X-ray repair cross-complementing group1 (*XRCC1*) forms protein complexes with DNA ligase III and DNA polymerase beta to repair gaps left during BER process (Wilson SH., 1998; Sancar A., 1994; Lindahl T. *et al.*, 1997). The *XRCC1* is a critical enzyme for this repair pathway and being alive. Mice lacking the *XRCC1* activity show a fatal phenotype (Wilson DM, 1997). The NER pathway primarily removes bulky DNA lesions from UV radiation or adducts produced by chemical carcinogens (Sancar A., 1994; Lindahl T. *et al.*, 1997). This process also involves a large number of proteins.

Defects in the NER pathway are known to be associated with three diseases, including xeroderma pigmentosum (XP), Cockayne's syndrome, and trichothiodystrophy (Sancar A., 1994; Benhamou S, 2000).

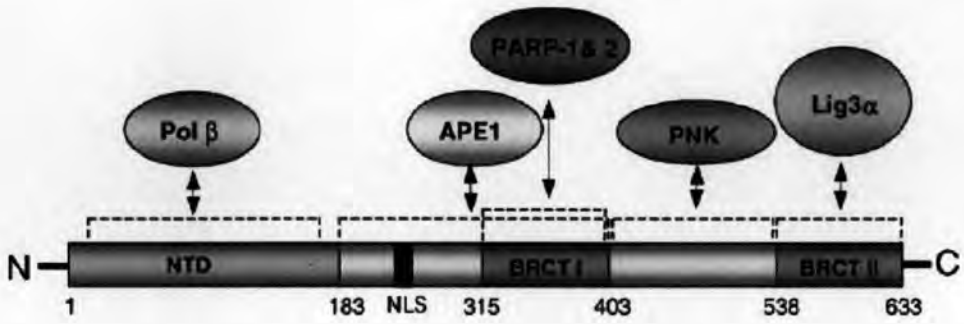
Figure 8 Base excision repair pathway (BER) (Keith W., 2003)



XRCC1 (X-ray repair cross complementing group 1)

X-ray repair cross complementing group 1 (*XRCC1*) was the first mammalian gene shown to play a role in cellular sensitivity to ionizing radiation (Thompson LH, *et al.*, 1990; Thompson LH., 2000). *XRCC1* located on 19q13.2. The construction of the *XRCC1* protein has been characterized to form repair complexes with DNA polymerase b (poly b) (Masson M, *et al.*, 1998), poly (ADP-ribose) polymerase (PARP) (Caldecott KW, *et al.*, 1996), and DNA ligase III (Caldecott KW, *et al.*, 1994).

Figure 9 Protein–protein interactions mediated by *XRCC1* (Caldecott KW, *et al.*, 1994).

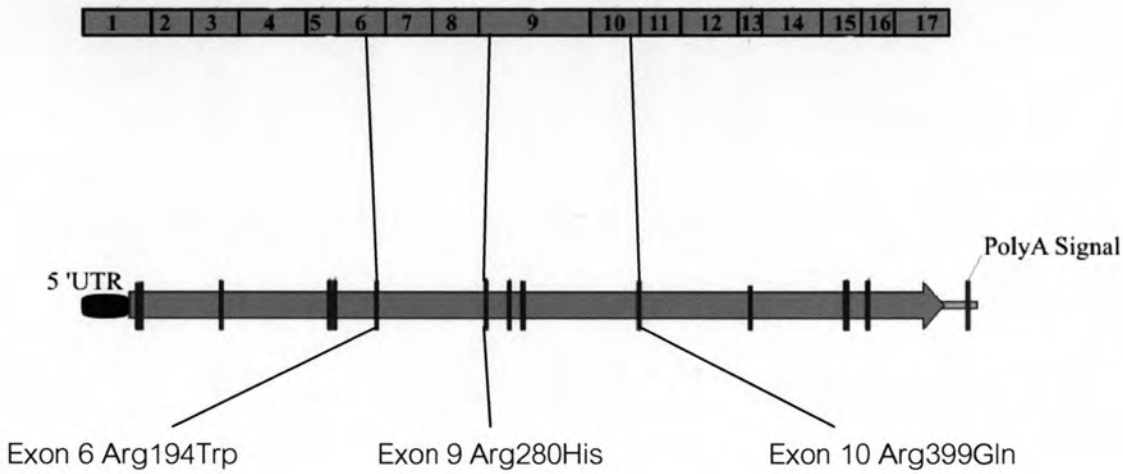


XRCC1 is thought to be involved in DNA single-strand beak (SSB) repair, and plays an important role in the base excision repair (BER) pathway. In orchestrating BER, *XRCC1* protein is proposed to interact with PARP and DNA ligase III in recognition and re-joining of DNA strand breaks (Masson M, *et al.*, 1998; Caldecott KW, *et al.*, 1994). And the ternary DNA-*XRCC1*-poly b complex has been suggested to act as "a scaffolding protein" (Masson M, *et al.*, 1998). Phenotype studies demonstrated that early embryonic lethality was associated with knockout of mouse *XRCC1* (Thompson LH, 2000). *XRCC1* mutants display sensitivity to alkylating agents and ionizing radiation and exhibit elevated levels of sister chromatid exchange in the Chinese hamster ovary cell lines (Thompson LH, *et al.*, 1990). Such alterations could be associated with increased cancer risk.

XRCC1 gene strand, three point mutants, Arg194Trp, Arg280His, and Arg399Gln, are amino acid substitution variants (Shen MR, *et al.*, 1998). Among these three mutations, we identified that 399Gln mutation might affect the *XRCC1* repair function through micronuclei assay in vitro (Qu T, 2005). Arg194Trp and Arg280His reside between the binding domains of poly b and PARP, while Arg399Gln is located in the vicinity of the PARP binding domain. More than one hundred research articles worldwide have reported the relationship of *XRCC1* polymorphisms (Arg194Trp, Arg280His, and Arg399Gln) and various cancer risks (table 3, table 4). Such environmental risk factors as ethnicity, gender, lifestyle and such genetic risk factors as

other genetic polymorphisms combined with the three polymorphisms of *XRCC1* might further increase the susceptibility to cancer.

Figure 10 Common *XRCC1* polymorphisms (http://scmgc.cmo.washington.edu/GeneModels/XRCC1.html).



In Asians the allele frequencies of the three *XRCC1* polymorphisms are different from Caucasians. The association of *XRCC1* polymorphisms and cancers susceptibility in Asians is also different from Caucasians (Shen MR, *et al.*, 1998). For practical future cancer prevention programs, we reviewed the relationship between the three *XRCC1* polymorphisms and cancer risks in all Asian population studies.

Table 3 Study characteristics and genotype prevalences from published studies on the relation of the x-ray repair cross-complementing group 1 (*XRCC1*) Arg194Trp and Arg280His polymorphisms to cancer risk (Rayjean J, 2005).

Cancer site or type	First author	Year	Ref. no.	Country	Ethnicity of subjects	No. of cases	No. of controls	Source of controls	Matching	Genotype 1/2 (heterozygotes)	Genotype 2/2 (rare-allele homozygotes)	Hardy Weinberg p value*
						XRCC1 Arg194Trp						
Lung	Ratnasinghe	2001	68	China	Asian	108	216	Population	Individual	48.1	9.7	0.26
	Chen	2002	69	China	Asian	109	109	Population	Individual	36.7	4.6	0.69
	David-Beabes	2001	23	United States	African-American	154	243	Population	Frequency	14.9	0.9	0.76
	David-Beabes	2001	23	United States	Caucasian	180	461	Population	Frequency	11.7	0.0	0.18
	Hung	2005	56	Europe	Caucasian, UEM‡	2,147	2,132	Hospital	Frequency	13.7	0.6	0.93
Upper aerodigestive tract	Lee	2001	70	Taiwan	Asian	105	264	Hospital	Frequency	45.5	7.2	0.17
	Xing	2002	71	China	Asian	433	524	Population	Frequency	43.5	7.1	0.16
	Yu	2004	41	China	Asian	135	152	Check up	Frequency	39.5	2.6	0.09
	Hao	2004	58	China	Asian	411	478	Population	Frequency	43.7	7.9	0.33
	Sturgis	1999	72	United States	Caucasian, UEM	203	424	Hospital	Frequency	14.4	0.0	0.11
	Olshan	2002	73	United States	Caucasian	98	161	Hospital	Frequency	16.1	0.0	0.27
	Varzim	2003	74	Portugal	Caucasian	88	178	Blood donor	No	10.1	0.0	0.48
Bladder	Stern	2001	28	United States	Caucasian, UEM	232	210	Hospital	Frequency	17.6	0.0	0.16
Colorectum	Abdel-Rahman	2000	75	Egypt	African	48	48	Friend	Individual	10.4	0.0	0.70
Stomach	Shen	2000	76	China	Asian	188	166	Population	Frequency	46.4	11.4	0.75
	Lee	2002	77	Korea	Asian	190	172	Hospital	Frequency	50.0	8.1	0.09
Prostate	van Gils	2002	78	United States	Caucasian, African-American	76	182	Population	Frequency	15.4	1.1	0.58
Breast	Duell	2001	24	United States	African-American	155	160	Population	Frequency	12.5	0.0	0.40
	Duell	2001	24	United States	Caucasian	233	221	Population	Frequency	13.1	0.9	0.45
	Han	2003	79	United States	Caucasian	998	1,369	Population	Frequency	12.9	0.2	0.18
	Smith	2003	80	United States	Caucasian	114	230	Clinic	No information available	15.7	0.4	0.62
	Smith	2003	81	United States	Caucasian	246	266	Hospital healthy	Frequency	8.6	0.4	0.57
	Moullan	2003	82	France	Caucasian	254	312	Blood donor	No	13.1	0.3	0.67
	Forsti	2004	83	Finland-Poland	Caucasian	223	298	Blood donor	Frequency	5.4	0.0	0.63
	Deligezer	2004	84	Turkey	Turkish	151	133	Healthy	No information available	10.5	0	0.52
	Kim	2002	32	Korea	Asian	205	205	Hospital	Individual	42.0	13.2	0.34
	Checko	2005	40	India	Asian	123	123	Hospital	Individual	18.7	3.3	0.09
Acute myeloid leukemia	Seedhouse	2002	27	Finland	Caucasian	114	87	No information available	No	8.0	2.3	<0.01
Skin	Winsey	2000	31	United Kingdom	Caucasian	125	211	Hospital healthy	No	17.1	0.0	0.18

Table 4 Study characteristics and genotype prevalences from published studies on the relation of the x-ray repair cross-complementing group 1 (*XRCC1*) Arg399Gln polymorphism to cancer risk(Rayjean J, 2005).

Cancer site or type	First author	Year	Ref. no.	Country	Ethnicity of subjects	No. of cases	No. of controls	Source of controls	Matching	Genotype 1/2 (heterozygotes)	Genotype 2/2 (rare-allele homozygotes)	Hardy-Weinberg p value*
Lung	Ratnasinghe	2001	68	China	Asian	107	208	Population	Individual	38.5	5.3	0.57
	Chen	2002	69	China	Asian	109	109	Population	Individual	36.7	6.4	0.87
	Park	2002	85	Korea	Asian	192	135	Hospital healthy	Frequency	35.6	4.4	0.74
	Ito	2004	65	Japan	Asian	178	449	Hospital	Frequency	37.6	5.8	0.76
	Zhang	2005	86	China	Asian	1,000	1,000	Hospital	Frequency	38.0	8.9	0.08
	David-Beabes	2001	23	United States	African-American	154	243	Population	Frequency	28.8	3.7	0.65
	David-Beabes	2001	23	United States	Caucasian	180	461	Population	Frequency	47.1	12.6	0.67
	Divine	2001	87	United States	Caucasian, Hispanic	172	143	Hospital	No	44.8	9.8	0.76
	Zhou	2003	88	United States	Caucasian	1,091	1,240	Hospital healthy	No	44.0	11.5	0.66
	Harms	2004	89	United States	Caucasian	110	119	Cancer-free	Frequency	46.2	6.7	0.26
	Misra	2003	66	Finland	Caucasian	315	313	Population	Individual	41.5	9.3	0.84
	Popanda	2004	67	Germany	Caucasian	463	460	Hospital	No	48.3	14.6	0.71
	Hung	2005	56	Europe	Caucasian, UEM‡	2,049	2,015	Hospital	Frequency	43.7	12.9	0.11
Upper aerodigestive tract	Lee	2001	70	Taiwan	Asian	105	264	Hospital healthy	Frequency	40.9	9.1	0.78
	Xing	2002	71	China	Asian	433	524	Population	Frequency	37.4	9.4	0.09
	Cho	2003	57	Taiwan	Asian	334	282	Community	Frequency	38.7	7.4	0.81
	Yu	2004	41	China	Asian	135	152	Check up	Frequency	38.8	3.3	0.19
	Hao	2004	58	China	Asian	411	479	Population	Frequency	41.1	6.9	0.48
	Sturgis	1999	72	United States	Caucasian, UEM	203	424	Hospital	Frequency	46.5	10.8	0.48
	Olshan	2002	73	United States	Caucasian	98	161	Hospital	Frequency	50.9	10.6	0.18
	Varzim	2003	74	Portugal	Caucasian	88	178	Hospital healthy	No	44.9	10.1	0.76
Bladder	Stern	2001	28	United States	Caucasian, UEM	233	210	Hospital	Frequency	45.7	12.4	0.98
	Kelsey	2004	29	United States	Caucasian, UEM	355	544	Population	Frequency	42.3	15.8	0.03
	Matullo	2001	42	Italy	Caucasian	124	84	Hospital	No	48.8	14.3	0.79
	Shen	2003	90	Italy	Caucasian	201	214	Hospital	Frequency	45.8	11.2	0.78
	Sanyal	2004	91	Sweden	Caucasian	311	246	No information available	Frequency	44.7	9.3	0.61
Colorectum	Abdel-Rahman	2000	75	Egypt	African	48	48	Friend	Individual	18.8	4.1	0.17
	Yeh	2005	92	Taiwan	Asian	776	736	Hospital	Frequency	39.5	7.3	0.99
Stomach	Shen	2000	76	China	Asian	188	166	Population	Frequency	35.5	7.8	0.39
	Lee	2002	77	Korea	Asian	190	172	Hospital	Frequency	34.3	5.2	0.87
Liver	Yu	2003	93	Taiwan	Asian	577	389	Hospital	Frequency	36.8	7.2	0.50
Pancreas	Duell	2002	30	United States	Caucasian, UEM	293	919	Population	Frequency	39.7	11.1	0.03
Breast	Duell	2001	24	United States	African-American	253	266	Population	Frequency	24.1	1.5	0.65
	Duell	2001	24	United States	Caucasian	386	381	Population	Frequency	41.5	15.5	0.05
	Han	2003	79	United States	Caucasian	986	1,337	Population	Frequency	46.1	13.2	0.93

Cigarette smoke

Cigarette smoke exposes children to many hazardous components. Cigarette smoke contains >4000 known and >100,000 unknown constituents and in cigarette smoking, a substantial amount of free radicals are introduced exogenously (C.J. Smith, 2001). The main hazard of smoking cigarettes is that it has been linked to major diseases, such as cancer, cardiovascular disease, emphysema, asthma, and stroke (Pryor, 1987).

Table 5 Primary Toxic and Carcinogenic components of Cigarette Smoke including vapour-phase and particulate phase components (C.J. Smith, 2001).

Agent	Toxic	Ciliotoxic	Carcinogenic	Co-carcinogenic / Promoter
Carbon Monoxide	X			
Nitrogen Oxides (NO_x)	X			
Hydrogen Cyanide	X			
Formaldehyde		X	X	
Acrolein		X		
Acetaldehyde		X		
Ammonia		X		
Hydrazine	X			
Vinyl Chloride Urethane			X	
2 - Nitropropane			X	
Quinoline			X	
Benzo[a]pyrene			X	

Table 5 Primary Toxic and Carcinogenic components of Cigarette Smoke including vapour-phase and particulate phase components (continue).

Agent	Toxic	Ciliotoxic	Carcinogenic	Co-carcinogenic / Promoter
Dibenz[a,h]anthracene			X	
Benzo[b]fluoranthene			X	
Benzo[j]fluoranthene			X	
Dibenzo[a,h]pyrene			X	
Dibenzo[a,i]pyrene			X	
Dibenz[a,j]acridine			X	
Indeno[1,2,3-cd]pyrenex			X	X
Benzo[c]phenanthrene			X	X
Benz[a]anthracene			X	X
Benzo[e]pyrene			X	X
Chrysene			X	X
Methylchrysene				X
Mehtylfluoranthene				X
Dibenz[a,c]anthracene				X
Dibenz[a,h]acridine				X
Dibenzo[c,g]carbazole				X
Mehtylnaphtalenes				X
1-Methylindoles				X
Dichlorostilbene				X
Catechol				X
3-Methycatechol				X
4-Methycatechol				X
4-Ethycatechol				X
4-n-Propylcatechol				X
Nitrosodimethylamine			X	
Nitrosoethymethylamine			X	

Table 5 Primary Toxic and Carcinogenic components of Cigarette Smoke including vapour-phase and particulate phase components (continue).

Agent	Toxic	Ciliotoxic	Carcinogenic	Co-carcinogenic / Promoter
Nitrosodiethylamine			X	
Nitrosodi-n-propylamine			X	
Nitrosodi-n-butylamine			X	
Nitrosopyrrolidine			X	
Nitrosopiperidine			X	
Nitrosomorpholine			X	
N'-Nitrosonornicotine			X	
4-(methylnitrosamino)-1-			X	
(3-pyridyl)-1-butanone			X	
N'-Nitrosoanabasine			X	
N'-Nitrosoanatabine			X	
Aromatic			X	
Amines			X	
Aromatic			X	
Polonium-210			X	
Nickel			X	
Arsenic			X	
Cadmium			X	

Cigarette smoke is a rich source of chemical carcinogens and reactive oxygen species (ROS). Chemical carcinogens include polycyclic aromatic hydrocarbons, aromatic amines and *N*-nitroso compounds, which can produce DNA bulky adducts that may lead to DNA damage (Vineis, P., *et al.,* 1996). ROS are present in both the gas-phase and the particulate matter (Pryor, 1997; Kiyosawa, H., 1990) and include oxygen radicals, e.g., superoxide radicals ($O_2^{\cdot}$) and hydroxyl radicals ($OH^{\cdot}$), and

some derivatives of O_2 that lack unpaired electrons, e.g., hydrogenperoxide (H_2O_2) and hypochlorous acid (HOC) (Halliwell, 1994). One report indicated that 5×10^4 radicals are generated with each inhalation from a cigarette (Kiyosawa, 1990). Furthermore, through endogenous enzymatic reactions mediated by bacteria and inflammatory cells, *N*-nitroso compounds, such as those in cigarette smoke, can generate nitric oxide radicals that can induce oxidative damage (Bartsch, 1992). The accumulation of ROS leads to oxidative stress, which is a risk factor for cancer development (Bankson, 1993). ROS can initiate lipid peroxidation, oxidize proteins, and cause damage to DNA indirectly or directly (Pryor, 1997; Joenje, 1989; Floyd, 1990). Indirect damage includes inactivation of target enzymes, such as those involved in DNA synthesis (Friedberg, 1995). Direct DNA damage includes DNA strand breaks, creation of abasic sites, and base adduct formation, such as thymine glycol, 5-hydrozymethyluracil and 8-hydroxy-2-deoxyguanosine (Friedberg, 1995). Up to 4×10^5 oxidatively altered DNA base residues are introduced per day in each cell (Roldan-Arjona, *et al.*, 1997). Bulky adduct lesions induced by chemical carcinogens are repaired through the nucleotide excision repair (NER) pathway (Benhamou, 2000). Base damage and DNA single strand breaks are repaired through the BER pathway (Wilson, 1997). This pathway is a multistep process that requires the activity of several proteins (Wilson, 1997).