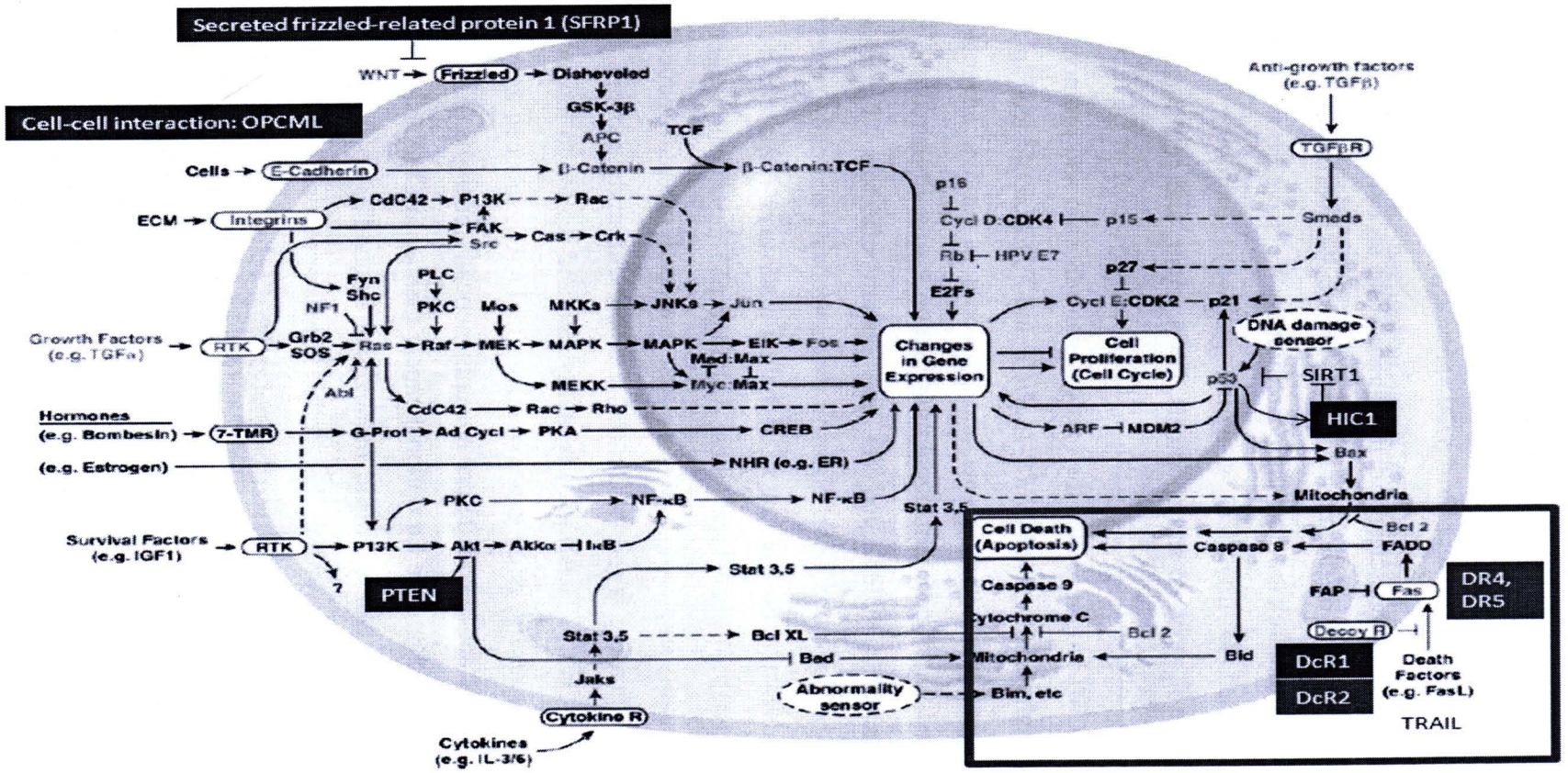
CHAPTER V
CONCLUSIONS AND STUDY REMARKS

The present study has clearly demonstrated that an epigenetic change through DNA methylation is one of the most common molecular aberrations besides genetic defects in liver fluke related-cholangiocarcinoma. We have revealed that several CpG-islands related to important signaling pathways including cell cycle and proliferation control, survival and apoptosis, DNA repair, and cell interaction system have been epigenetically inactivated through hypermethylation in CCA. For instance, the silenced *OPCML* might reduce the intercellular adhesion and promote cancer cell invasion. The highly methylated *SFRP-1*, *HIC1* and *PTEN* could lead to an increase of proliferation, inhibition to apoptosis, and promotion of survival advantage in CCA through increased Wnt/β-cantenin signal transduction via the impaired p53-responsive pathway and activated PI3K and its downstream effectors. These mentioned aberrations are the notorious mechanisms in carcinogenesis and pathogenesis of CCA which can be drawn a conclusion of signaling pathways (Figure 5-1) adversely affected by epigenetically silenced genes. Although p53-dependent programmed cell death is epigenetically impaired, it is interesting that death receptor-induced apoptosis through TRAIL signaling is rarely silenced. Our present study showed that an anti-apoptotic receptor DcR1 (TRAIL-R3) is methylated and related to longer survival of CCA patients. Conversely, other genes involved in TRAIL-induced apoptosis (DR4, DR5, DcR2, CASP8 and APAF-1) are not epigenetically changed (Figure 5-2). Our findings suggest the advantage for optional cancer therapy in CCA using recombinant TRAIL or TRAIL receptor agonistic monoclonal antibodies to induce cancer cell death. Interestingly, it is a worth to therapeutic induce apoptosis via TRAIL signaling cascade as it is selectively kill cancer but not normal cells [184-186]. Moreover, the combined treatments of TRAIL therapy and standard chemotherapeutic drugs may increase CCA cell death and further reduce the chance of TRAIL resistance.



Modified from Hanahan and Weinberg, 2000

Figure 5-1 Scheme of the emergent integrated circuit of the cell (modified from [16]).

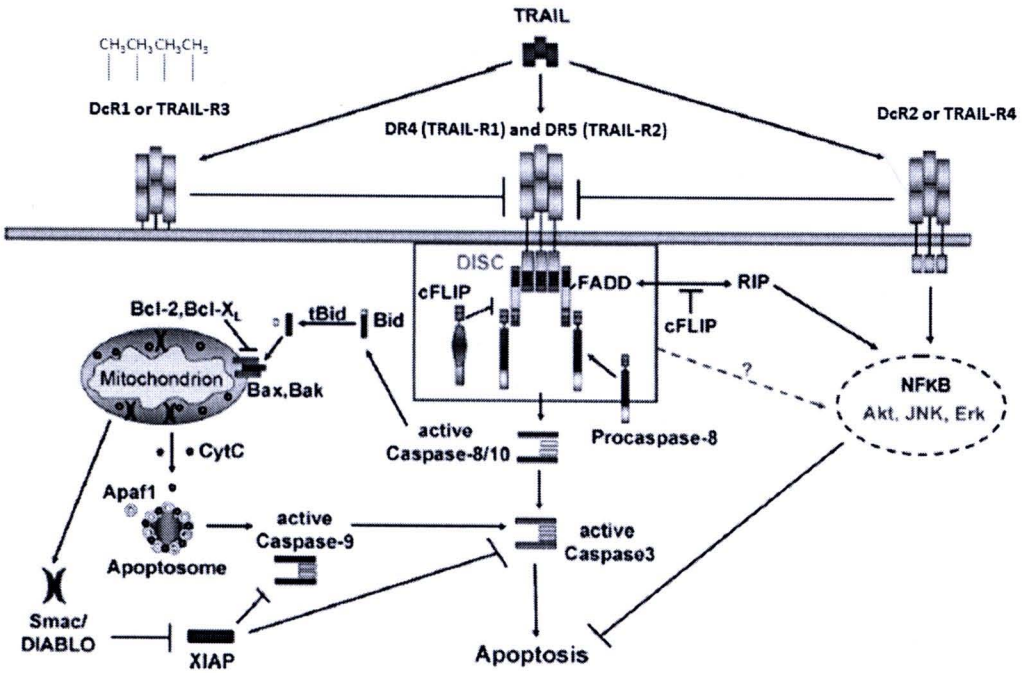


Figure 5-2 Scheme of the TRAIL signaling pathway. The increased methylation was found in *DcR1* (*TRAIL-R3*) but not other *TRAIL* cascade related genes indicates the use of recombinant TRAIL or TRAIL receptor agonistic monoclonal antibodies for selectively killed tumor cells (modified from [184]).

Regarding our finding in the high frequency of methylation in certain loci, it raised our interest to inspect the genome-wide DNA methylation signature specific to CCA. As our expectation, DNA methylation is dramatically changed in the genome-wide scale with hundreds of hypermethylation and hypomethylation observed. In our study, a number of differentially hypermethylated CpG sites have been found at genes that are associated with specific functional term of target genes of the Polycomb group (PcG) complexes including homeobox genes, EED target, PRC2 targets, SUZ12 targets, and H3K27 target genes. Stem cell PcG targets has been previously reported to be up to 12-fold more likely to have cancer-specific promoter DNA hypermethylation than non-targets. These PcG target genes might be permanently silenced leading to loss of differentiation properties of the cells [187]. Moreover, we found the hypomethylation is significantly associated to key regulator of embryonic

stem cell (ES) identity, especially OCT4 and NOS targets which might lead to gene overexpression. These genes are proposed as transcription factors in maintaining the self-renewal phenotype and usually overexpressed in ES cell [188-89]. Taken together, our finding supports the theory of stem cell origin in cancers, including CCA. Therefore, the biological functions of these candidate DNA methylation changes should be further clarified.