CHAPTER I

INTRODUCTION

Background and Rationale

Cervical cancer (CC) on a large scale causes death in women worldwide(1). High risk types of Human Papillomavirus (HPV) are the main etiological agents of CC (2,3). HPV-induced CC follows a multistep cancer development process (4). Specific histological classifications help differentiate between cervical premalignant and malignant lesions as low-grade and high-grade squamous intraepithelial lesions (SILs) and invasive cancers (5). Nonetheless, HPV infection alone cannot cause carcinogenesis. Whereas persistent HPV infection induces the development of SILs due to the viral genome encoding proteins for cellular proliferation and apoptosis evasion, accumulation of mutations as well as epigenetic alterations, particularly aimed at activating oncogenes and inactivating tumor suppressor genes, are crucial for tumor transformation from premalignant lesions to invasive cancers(6).

DNA double strand breaks (DSBs) can be caused by ionizing radiation or other DNA damaging agents or by normal DNA metabolic processes (7,8). After DSB formation, the cell cycle is arrested, followed by repair. If repairing mechanisms is failed or have erroneous DSB repair, this leads to genetic instability, gross chromosomal rearrangements and accumulation of mulations (7,9). These events then trigger the cell

cycle checkpoints resulting in permanent growth arrest or death of affected cells, otherwise if the checkpoints are inactivated by mutations, this lead to tumorigenesis (9).

Better understanding of repairing protein in molecular carcinogenesis will lead to improvement in both prevention and treatment of CC. The aim of this thesis has been to evaluate the potential of cyclin A1 (*CCNA1*) to act as a tumor suppressor gene in HPV-associated CC. *CCNA1* is a second A-type cyclin essential for entry into the metaphase of male meiosis I (10,11). In addition to testis, hematopoietic progenitor cells and acute myeloid leukemia, *CCNA1* is expressed at low levels in most other tissues (12). No phenotype other than male infertility has been reported in mice lacking *CCNA1* (13). Surprisingly, recent evidence suggests that *CCNA1* may be an epithelial tumor supressor gene. First, *CCNA1* is not only transcriptionally induced following *TP53* activation but the gene has also been demonstrated to be downregulated in several cancers such as nasopharyngeal carcinoma and head and neck squamous cell cancer (HNSCC) (1,14,15). Second, CCNA1 has been proven to exhibit a novel function in DNA double strand breaks (DSBs) repair following radiation damage by activating nonhomologous end joining (16). Base on the data of identification Ku70 DNA repair protein as a binding partner and substrate of the cyclin A1-CDK2 complex and DNA double strand break repair was deficient in *CCNA1*$^{-/-}$ cells. Finally, the promoter, similar to several key tumor suppressor genes, is frequently hypermethylated in colon cancer and HNSCC (1,17).

Moreover, based on the HNSCC study, a unique inverse relationship between *CCNA1* promoter methylation and *TP53* mutation status in primary tumors has been demonstrated (1). Similar to HNSCC, the majority of CC is of squamous cell origin and its molecular carcinogenesis strongly correlates with impaired TP53 function (18-20). However, unlike HNSCC, the functional loss of *TP53* in CC is not ascribed to gene mutation but processed by viral and host protein-protein interaction. CC is strongly associated with infection by high-risk HPV types and its oncoprotein E6 has the ability to associate with and neutralize the function of TP53 (19,20). E6 binds to TP53 and catalyzes multi-ubiquitination and degradation of TP53. Consequently, the majority of cervical cancer cells have a wild-type *TP53*, but the protein levels are decreased. Therefore, in comparison with HNSCC, it will be interesting to determine if *CCNA1* is methylated in HPV- associated squamous cell CC.

Objective

1. To study methylation status of *CCNA1* in cervical cancer, normal cervical tissue and blood.
2. To study the association between promoter methylation of *CCNA1* and gene expression.
3. To study the relationship between *CCNA1* promoter hypermethylation, HPV and clinical parameters.
4. To study methylation status of *CCNA1* along cervical carcinogenesis.

Research Question

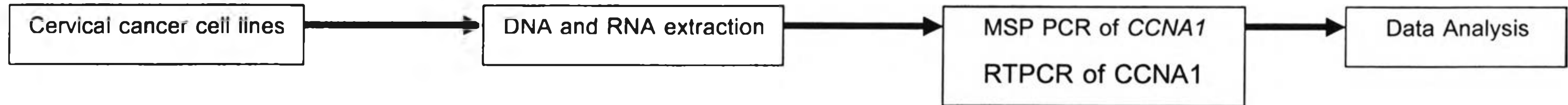
1. What are patterns of *CCNA1* promoter hypermethylation in normal cervix and CC tissues?
2. Whether the promoter methylation of *CCNA1* control the gene expression?
3. Whether the promoter methylation of *CCNA1* related to HPV and clinical parameters?
4 How the *CCNA1* promoter hypermethylation changes during multistep cervical cancer development?

Hypothesis

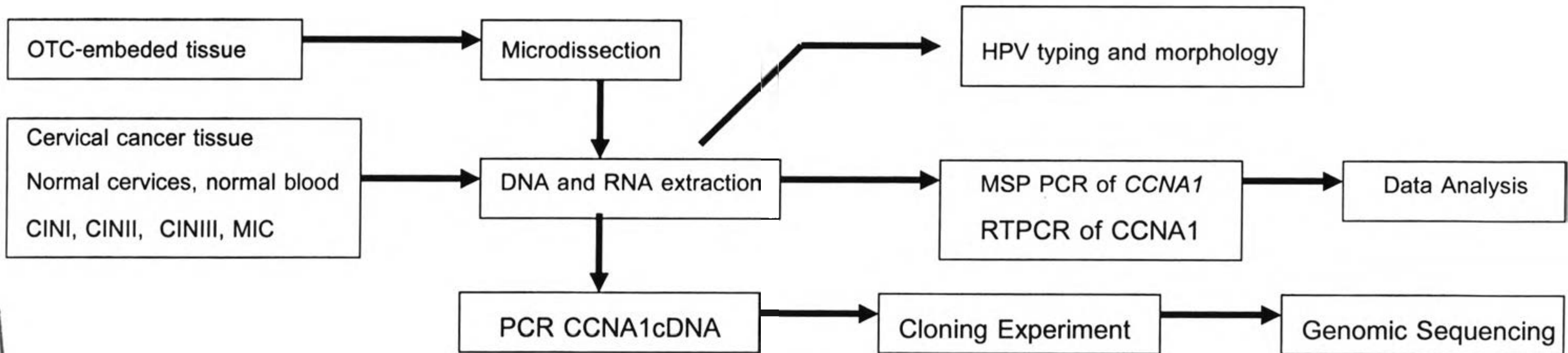
1. Promoter hypermethylation of *CCNA1* is found in cervical cancer.
2. Demethylation of promoter of *CCNA1* is found in normal epithelium and blood.
3. The *CCNA1* promoter methylation of cervical cancer plays a role in controlling gene expression *in vitro* and *in vivo*.
4. The *CCNA1* promoter methylation of cervical cancer related to HPV and clinical parameter.
5. Different level of hypermethylation of *CCNA1* is presented during different stages of cervical carcinogenesis.

Conceptual framework

In vitro experiment



In vivo experiment



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Expected benefit

The results of this study will be benefit in cervical carcinogenesis molecular biology and the promoter hypermethylation of *CCNA1* may lead to serve as a convenience tumor marker and developing new, effective targeted therapies.

Research methodology

1. Normal cervical tissue, cervical cancer tissue and blood collection.
2. Tissue culture, paraffin embedded and frozen embedded tissue preparation.
3. Microdissection materials from frozen embedded tissues.
4. Investigation of HPV typing and morphology.
5. DNA and RNA extraction.
6. Sodium bisulfite treatment and desalted with DNA clean-up system.
7. Primer design for *CCNA1* gene.
8. PCR amplification of
   - the MSP of *CCNA1*
   - the RTPCR of *CCNA1*
9. Gel eletrophorhesis, quantitation with Molecular Dynamics phosphoimager.
10. Cloning and Sequencing.
11. Data collection and analysis.