CHAPTER I

BACKGROUND

Notch genes encode heterodimeric transmembrane receptors that regulate differentiation, proliferation and apoptosis of various cell types in vertebrates (Artavanis-Tsakonas *et al.*, 1999). The mammalian family of Notch receptors consists of four Notch proteins (Notch1-4) and five ligands, i.e. Jagged1, Jagged2, Delta-like1 (Dll1), Dll3 and Dll4. Upon receptor-ligand interaction, the Notch receptors are subjected to proteolytic processing. The first cleavage is mediated by TACE (tumour-necrosis factor-α-convering enzyme/metalloproteinase), which is followed by a second cleavage by presenilins (PS) containing enzyme complex called the γ-secretase. The intracellular domain of Notch (ICN) is released from the cell membrane and enters the nucleus, where it binds to a transcription factor CSL (CBF-1 in mammals, suppressor of hairless in *Drosophila* and LAG-1 in *Caenorhabditis elegans*), leading to transcriptional activation of downstream target genes. One of the best characterized Notch targets is the HES (hairy/enhancer of split) family of transcription factors (Radtke and Raj, 2003). Mastermind-like (MAML) proteins family are required for Notch signaling (Wu and Griffin, 2004). MAML forms a ternary complex with CSL-ICN via a direct interaction with ICN. Under normal condition, Notch signaling plays a key role in the differentiation of various types of cells. Aberrant Notch signaling, however, is linked to cancers, such as T-cell acute lymphoblastic leukemia and breast cancer (Ellisen *et al.*, 1991, Stylianou *et al.*, 2006). Notch signaling is shown to function both to suppress and promote tumor formation in different settings. Several factors may play a role in determining whether Notch acts to promote or suppress tumorigenesis (Leong and Karsan, 2006).

Infection by high-risk human papillomavirus (HPV) (such as HPV type 16 , HPV-18, and HPV-31) and continued expression of the viral *E6* and *E7* genes are linked to the progression of human cervical cancers, a major subset of neoplasia in women worldwide (zur

Hausen, 1996). Several reports indicated that Notch signaling is associated with HPV cervical cancer, but the precise role of this signaling is still controversial.

Oncogenic functions of Notch signaling in cervical cancer are examplified by the report showing that expression of high levels of Jagged1 is linked to Notch activation in the cervical tumor-derived cell line CaSki (Veeraraghavalu *et al.*, 2004). In other report, activated Notch1 inhibits p53-induced apoptosis in HaCaT cells. This function is dependent on a PI3K-PKB/Akt pathway (Nair *et al.*, 2003b). Another cell signaling pathway that has been shown to be disregulated in human cervical cancers is NF-κB (Nair *et al.*, 2003a, Prusty *et al.*, 2005). Both Notch and NF- κB are coexpressed and colocalized in HPV-positive CaSki cells. NF-κB is likely to be downstream of the Notch signaling (Ramdass *et al.*, 2007). Yu *et al.* reported that blocking Notch signaling inhibited the growth of cervical cancer cell line, HeLa, both *in vitro* and *in vivo* (Yu *et al.*, 2007a). In addition, they found that downregulation of Notch1 by RNAi suppressed the proliferation of HeLa (Yu *et al.*, 2007b).

In some reports, Notch is a tumor suppressor in cervical cancer. Talora *et al.* showed that high levels of Notch1 protein was detected in early-stage of cervical tumorigenesis, whereas late-stage cervical cancers had reduced Notch1 protein levels (Talora *et al.*, 2002). It is also reported that in cervical cancer cell lines, activated Notch1 inhibited cell cycle progression which implied that activation of Notch signaling in cervical cancer cells resulted in cell cycle arrest of cervical cancer cells (Talora *et al.*, 2005). Consistently, Yao *et al.* found that activated Notch1 resulted in growth inhibition of HeLa (Yao *et al.*, 2007) and overexpression of ICN significantly inhibited *in vitro* growth of cervical carcinoma cells (Wang *et al.*, 2007). Thus, Notch1 signaling plays a key role during HPV-induced carcinogenesis and exerts dual effects, functioning in a context-dependent manner.

The MAML family, mammalian counterparts of the *Drosophila* protein mastermind, was identified by Wu *et al.* (Wu *et al.*, 2002). This protein in this family appears to functions specifically in Notch signaling and as co-activator of p53 (Zhao *et al.*, 2007). The human MAML family consists of three members, MAML1, MAML2 and MAML3 (Wu *et al.*,

2002). The three human MAMLs each contain an N-terminal basic domain and two acidic domains (I and II) in the middle region and the C-terminus. MAML directly bind to the most conserved domain of all four Notch receptors, ankyrin repeats, through their N-terminal conserved basic domains. They interact with CSL only in the presence of ICN, forming a stable DNA-binding ternary complex. MAML activate transcription of Notch target genes when Notch receptors are stimulated by ligand engagement, or when constitutively active forms of Notch ICN are expressed (Wu and Griffin, 2004). Therefore, MAMLs are essential regulatory proteins in Notch signaling.

MAML are linked to several types of cancer such as mucoepidermoid carcinoma (MEC), T cell leukemia and cervical cancer (Wu and Griffin, 2004). In addition, it was first cloned as a binding protein for high-risk HPV type 16 E6 in a yeast two-hybrid screening (Wu *et al.*, 2000). The preliminary studies indicated that transforming variants of E6 interacted with human MAML and led them to proposed that these interactions may possibly interfere with the functions of MAML proteins.

Even though the involvement of Notch signaling in cervical cancer progression is well established, the exact role of Notch signaling in HPV cervical cancer remains controversial. On the basis of these observations, we utilized γ-secretase inhibitor and DN-MAML1 (dominant negative mastermind-like1) that contains truncated MAML1 (corresponding to amino acid of 12-74 from an N-terminus) fused to GFP, to suppress Notch signaling and examined the effects of this suppression on phenotypes of HPV-positive cervical cancer cells.

Objective

The purpose of this research is to study the effects of suppressing of Notch signaling in HPV-positive cervical cancer cell lines, using pharmacological inhibitor and genetic (DN-MAML1) approaches on the phenotypes of these cell lines.

Research framework

1. Study mRNA expression of *Notch receptor*, *Hes-1*, *MAML1*, *E6*, *E7* and *p53* by RT-PCR or Real-time PCR and protein expressions of Notch1 and cleaved Notch1 by Western blot.
2. Study cell viability and cell cycle by MTT assay and cell cycle analysis.
3. Determine cell proliferation by clonogenic assay.