Stat-3 and the invasion of cervical cancer cell relationship

CHAPTER 1

INTRODUCTION

Cervical cancer is one of the most highly leading cancers among the women in the world. The estimated number of cervical cancer cases and death in the world is increase to 527, 624 and 265, 653 respectively in year 2012 (Saranath & Khanna, 2014). This statistic had influenced the society to be more aware about cervical cancer and learn how to lower the possibility of getting this disease. The main causes of cervical cancer are due to the infection of Human Papillomavirus (HPV) (Wheeler, 2007). However, HPVs alone is inadequate to cause malignant changes (Martin, Astbury, & O'Leary, 2006). It also depends on other genetic factors to lead to tumorigenic conversion of cervical tissues. Smoking, poverty, Human Immunodeficiency Virus (HIV), Chlamydia infection can also lead to development of cervical cancer.

In this study, we wanted to investigate the relationship between signal transducer and activator of transcription 3 (STAT-3) and the invasion of cervical cancer cells. Based on the evidence that STAT-3 is constitutively activated during development and metastasis of cervical cancer cell, it is suspected to involve in the invasion, proliferation, apoptosis and metastasis of cervical cancer cell. Therefore, the objective of this study is to knockdown the STAT-3 gene by using RNA interference (RNAi) method. Subsequently, growth pattern and many different aspect of the cervical cancer cell can be observe to evaluate the relationship between STAT-3 and the invasion of cervical cancer cell using 3-dimensional spheroid.

CHAPTER 2

LITERATURE REVIEW

2. 1 ME-180 cervical cancer cell line

ME-180 cervical cancer cell line is derived from homo sapiens' cervix tissue especially from the metastatic site which known as omentum. Omentum is made up of two layers of peritoneum that cover and protect the abdominal organs. ME-180 cell line is anchorage-dependent which require a solid substratum for attachment before growth can occur. Anchorage-dependent cells rely on the electrostatic force and van der Waals' force interaction between the cell membrane and growth surface. Cell attachment occurs by divalent cations and basic proteins forming a layer between solid substratum and cell membrane. Therefore, this cell needed to be cultured on a suitable substrate that is specifically treated to allow cell adhesion and spreading. ME-180 cell line has an epithelial-like shaped morphology. Epithelial-like cells are polygonal in shape with more regular dimension and grow attached to a substrate in discrete patches. Karyotype of ME-180 cell lines range from hyperdiploid to hypohexaploid with abnormalities including dicentrics, fragmentation and markers. This cell line was originated from an invasive squamous cell carcinoma with uneven cell clusters and no significant keratinisation was observed. The properties of the cells in the monolayer culture when reach confluency are presence of cytoplasmic tonofibrils and desmosomal attachments between cells. In history, ME-180 cell line was detected to be contaminated by Mycoplasma arginini and successfully been eliminated in 1970 with the invention of 0. 1 micrometer pore filter. On the other hands, tumor necrosis factor alpha (TNF alpha) was determined to be

involved in the inhibition of the growth of ME-180 cell line. ME-180 cell line contains human papillomavirus (HPV) DNA with greater homology to HPV-39 than HPV-18. The gene expressed in ME-180 cell line including oncogene p53 and pRB which responsible for tumor suppressor gene (American Type Culture Collection).

- 2. 1. 1 Mycoplasma contamination in cell culture

One of the major problems in generating continuous cell line for research purpose is Mycoplasma contamination in cell culture. The first name given to Mycoplasma was pleuropneumonia-like organisms (PPLO) which has similarity to causative agent of contagious bovine pleuropneumonia (EDWARD, 1955). Mycoplasma is a smallest bacteria with a characteristic of lacking a rigid cell wall unlike other prokaryotes (S Razin & Freundt, 1984). Therefore, mycoplasma is placed under the separate class of Mollicutes (Tully & Razin, 1992). Mycoplasma is resistant to antibiotic which target cell wall synthesis because it does not possess any cell wall. Mycoplasma cell has a diameter of 0. 3 to 0. 8 micrometer (Rottem & Barile, 1993). Due to their small size, Mycoplasma cell membrane has a high flexibility to pass through conventional microbiological filter. Furthermore, Mycoplasma has the ability of self-replication to generate and increase it population density (Shmuel Razin, Yogev, & Naot, 1998). Mycoplasma cell contains only important organelles for survival and reproduction like plasma membrane, ribosomes, and a circular double stranded DNA genome (Shmuel Razin, 1996). Compared to other bacteria, Mycoplasma cell has a longer generation time and thus grow slowly under optimal conditions (Waites & Talkington, 2004).

Lag phase of Mycoplasma growth is longer and therefore it may take more than one week to obtain visible colonies on cell culture.

The main causes of Mycoplasma contamination are inexperienced or distracted lab technician in handling the cells and reagents. Repeating use of same pipette during re-feeding operation and laboratory equipments in contact with Mycoplasma-infected cell can also increase the chances of Mycoplasma contamination. Mycoplasma-infected cell lines are most critical source for further spreading the contamination. When the cell cultures are stored closely together with Mycoplasma-infected cells, the percentage of persistence spreading is increases. The reason is because the droplet formation when handling cell culture contains high concentration of Mycoplasma from infected culture and prolonged survival of dried Mycoplasma (Drexler & Uphoff, 2002). Finally, cell samples from other colleagues and labs with inauthentical proven source will increase the chances of Mycoplasma contamination.

The effect of Mycoplasma contamination on cell culture is alteration of cellular metabolism and level of protein, RNA and DNA. Mycoplasma contamination can also introduce chromosomal aberration and modify host cell plasma membrane antigens. Nevertheless, Mycoplasma contamination influence signal transduction process and promote virus propagation and cellular transformation (Drexler & Uphoff, 2002). Furthermore, the growth and viability of the host cells is halted by Mycoplasma contamination. Lastly, prolonged Mycoplasma contamination leads to cell death.

2. 2 SiHa cervical cancer cell line

SiHa cervical cancer cell line is derived from homo sapiens' cervix tissue. SiHa cell line is anchorage-dependent which require a solid substratum for attachment before growth can occur. Therefore, this cell needed to be cultured on a suitable substrate that is specifically treated to allow cell adhesion and spreading. SiHa cell line has an epithelial-like shaped morphology. SiHa cell line is a human hypertriploid cell line with modal chromosome number 71 occuring in 24% of cells. The remaining percentage consists of cells with chromosome number range between 69 to 72. 7. 6% of the cell line is polyploid cells. There is presence of marker chromosome observed in the SiHa cell line. Balanced translocation between two N2s produces dup(2) (q22q31) and del(2) (q31). Majority of SiHa cells had two copies of del(2). M2 is an A3-sized acrocentric while M13 is a minute submetacentric with 1-3 copies per cell. Sources of both M2 and M13 are not determined. Two copies of normal X chromosomes are present in SiHa cell line. The absence of N2 is probably replaced by dup(2) and del(2). SiHa cell line is derived from fragments of a primary tissue sample obtained after surgery from a Japanese patient. Presence of typical desmosomes was observed under electron microscope at the cell junctions. In addition, abundance of tonofilaments was also observed in the cytoplasm. Mycoplasma contamination was detected and eliminated in 1975. The SiHa cell line is reported to contain an integrated human papillomavirus type 16 genome (HPV-16, 1 to 2 copies per cell). The gene expressed in ME-180 cell line including oncogene p53 and pRB which responsible for tumor suppressor gene (American Type Culture Collection).

2. 3 STAT family

STAT is transcription factor that function to regulate the cell's growth, proliferation, apoptosis, survival, differentiation, immune response and metastasis. There are 7 types of different STAT protein which include STAT 1, 2, 3, 4, 5A, 5B and 6 (Ihle, 2001). Src-homology 2 (SH2) domain play an important role for the activation of STAT protein by binding to phosphptyrosine. Janus kinases (JAKs) function to activate the STAT transcription factor. JAK-STAT signaling pathway is shown to be enhanced the survival, immunosuppression and angiogenesis of primary tumors. Cytokines and growth factors which act as ligand bind to specific receptor like interferons IL-5, IL-6 and epidermal growth factor receptor (EGFR) (Dimberg et al., 2012; Grandis et al., 1998). This subsequently causes these receptors to dimerize to form a dimer and start to recruit JAKs. The polymerization of JAKs causes self-stimulation by either auto- or trans-phosphorylation and phosphorylate the tyrosine residues on the cytoplasmic domain of receptor. Anchorage site which represent by the phosphotyrosine on the receptor will bind to the STAT's SH2 domain and lead to the phosphorylation of STAT proteins at specific tyrosine residues in the C-terminal domain. Consequently, the activated STAT proteins will form homo- or hetero- dimers which will translocate to the nucleus via importin alpha/beta and RanGDP complex. Next, it will bind to specific sequences on the cytokine-inducible promoters of target genes which contain gamma-activated site (GAS) motif to initiate the gene transcription (Rane & Reddy, 2000). The inactivation of STAT is regulated by dephosphorylation using nuclear phosphatases which

subsequently causes the STAT to transport out of the nucleus via exportin crm1 or RanGTP.

- 2. 3. 1 STAT-3

STAT-3 is transcription factor that function to regulate the cell's growth, proliferation, apoptosis, survival, differentiation, immune response and metastasis. STAT-3 is located on chromosome 17q21. 31. Like other STAT family proteins, STAT-3 contains a dimerization domain at N-terminus, coiled-coil domain for protein-protein interactions, central DNA binding domain, SH2 domain for the recruitment to receptor, conserved tyrosine Ser-727 phosphorylation play a vital role for STAT-3 to be functional (Frank, Mahajan, & Ritz, 1997). Ser-727 phosphorylation can act as inhibition to suppress Tyr-705 residue at location 705 (Tyr-705), and a C-terminus encoding the transcription activation domain (Darnell, 2002; Rane & Reddy, 2000; Yu & Jove, 2004). Tyr-705 phosphorylation and phosphorylation (Chung, Uchida, Grammer, & Blenis, 1997). Dephosphorylation of STAT-3 at Tyr-705 using the tyrosine phosphatases in the cytoplasm is also another ways of inhibition (Zhang et al., 2009). Moreover, suppressors of cytokine signaling (SOCS) can also negative regulate JAK-STAT signaling pathway by binding to JAKs or competing with STATs for the anchorage site on the cytokine receptors (Krebs & Hilton, 2001). Lastly, JAK-STAT signaling pathway can also be negative regulated by protein inhibitors of activated STAT (PIAS) in the nucleus by blocking the DNA sequences to inhibit the transcriptional activation of STAT (Shuai, 2006).

2. 4 HPV

HPVs are made up of small, non-enveloped viruses with circular double stranded DNA (Sanclemente & Gill, 2002). There are many HPV strains that cause the growth of various types of human cancers. The most dangerous widespread strain is HPV16 and HPV18 (Zur Hausen, 2002), both are known as “ high risk” HPV types. Around 70% of cervical cancer cases are due to infection of HPV16 and HPV18 (Boshart et al., 1984; Dürst, Gissmann, Ikenberg, & Zur Hausen, 1983). The genome of the virus made up of 7200-8000 base pairs and consists of three segments (Castro & Bussoloti Filho, 2006). The three segments include early region (E1, E2, E4-7), late region (L1 and L2) and genomic regulatory region. Early region is responsible for regulation of DNA replication (E1, E2), transcription (E2), cell transformation (E5, E6, E7) while late region is coded for structural protein of the virion (Devaraj, Gillison, & Wu, 2003). HPV infection is also promoted by the inactivation of tumor suppressor gene or activation of cellular oncogenes which lead to cervical cancer development. One of the examples is constitutive expression of E6 and E7 viral oncogenes. The proteins produced by these oncogenes interact with p53 and pRB proteins causes cell cycle to be interrupted (Boyer, Wazer, & Band, 1996; Huibregtse, Scheffner, & Howley, 1991). Finally, E6 and E7 viral oncogenes also promote deregulation of proliferation and genetic variation which lead to mutation and aneuploidy state (Duensing & Münger, 2002).