ADAM-9 EXPRESSION IN SQUAMOUS CELL CARCINOMA, ADENOCARCINOMA AND ADENOSQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

DR SEOPARJOO AZMEL BIN MOHD ISA

MASTER OF PATHOLOGY (ANATOMIC PATHOLOGY)

Jabatan Patologi,

Pusat Pengajian Sains Perubatan

Universiti Sains Malaysia

16150 Kota Bharu

Kelantan

Introduction: Cervical cancer is a preventable and the third most common cancer to occur in the reproductive age women. It is caused by the Human Papilloma Viruses. The ADAM 9 protein is involved in basement membrane degradation and tumor metastasis in certain types of tumor.

Objective: Objectives of this study are to describe the characteristics of cervical cancer and factors associated with ADAM 9 expression in cervical cancer.

Methodology: Retrospective record review was done in February 2012 using patients' case notes obtained from the Medical Record Unit, Universiti Sains Malaysia Hospital. Data was entered and analyze using SPSS version 19.

Result: There were 95 cases of cervical cancer included in this study. Majority were Malays 76 (80.0%), non smoker 85 (89.5%), without co-morbid disease (86.4%), had no distant metastasis 54 (56.8%) and absent of lymph nodes involvement 71 (74.7%). Squamous cell carcinoma was the most common type of cervical cancer 67 (70.5%). Mean age of subjects were 53.89 (10.83). The statistically significant associated factors for ADAM9 positive staining among

cervical cancer were tumor size (p=0.004), distant metastasis (p=0.009) and histological type of cervical cancer, which is squamous cell carcinoma (p=0.017). Women with squamous cell carcinoma of cervical cancer has 7.39 times odds of having ADAM9 staining positive as compared with women with adenocarcinoma of cervical cancer (95% CI 1.42, 38.51; p=0.017). Cervical cancer patients who had distant metastasis has 12.82 times chances of having ADAM9 staining positive as compared with cervical cancer patients without distant metastasis (95% CI 1.91, 86.13; p=0.009). while with every 1mm increment in tumor size, women with cervical cancer will had 1.08 odds times od having ADAM9 staining positive (95% CI 1.02, 1.13; p=0.004).

Conclusion: In this study we identified that ADAM 9 expression associated with large size of the tumor, lymph nodes involvement, distant metastasis and also type of carcinoma. This concludes that aggressive tumor expressed ADAM 9.

DR MD SALZIHAN BIN MD SALLEH: SUPERVISOR

DR MOHD PAZUDIN BIN ISMAIL: CO-SUPERVISOR

ADAM-9 EXPRESSION IN SQUAMOUS CELL CARCINOMA, ADENOCARCINOMA AND ADENOSQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

BY

DR SEOPARJOO AZMEL BIN MOHD ISA

Dissertation submitted in partial fulfilment of the requirement for the degree of Master of Pathology (Anatomic Pathology)

2012

Acknowledgement

In the name of Allah. First and foremost, my highest thanks and gratitude to Allah. Under His power I manage to finished my dissertation. I also would like to express greatest thanks to few peoples who help me during my dissertation.

1. Dr Md Salzihan bin Md Salleh

   Main Supervisor,

   Lecturer and Pathologist, School of Medical Sciences,

   Universiti Sains Malaysia

2. Dr Mohd Pazudin bin Ismail

   Lecturer and Obstetric & Gynecology Specialist, School of Medical Sciences,

   Universiti Sains Malaysia

3. Staff at pathology lab, especially Cik Rohaida and En Rosli

4. To all my lecturers in Pathology department

5. My dearest wife, Dr Suhaily binti Mohd Hairon and son, Eaman Danish bin Seoparjoo Azmel.

Table of Contents

List of tables and figures

Abstrak

Kanser serviks adalah sejenis kanser yang boleh dicegah dan kanser ketiga paling kerap berlaku dikalangan wanita. Ianya adalah disebabkan oleh Virus Papilloma Manusia. Protein ADAM 9 berfungsi menguraikan membran dasar dan juga terlibat dalam rebakan sel-sel kanser bagi sesetengah jenis kanser. Objektif kajian ini dijalankan adalah untuk mengenalpasti ciri-ciri yang berkaitan dengan ekspresi protein ADAM 9 di dalam kanser serviks. Semakan rekod retrospektif dilakukan pada bulan februari 2012 melalui nota pesakit di Unit Rekod Perubatan, Hospital Universiti Sains Malaysia. Data dimasukkan dan dianalisa menggunakan SPSS versi ke-19. Terdapat 96 kes kanser serviks dimasukkan ke dalam kajian ini. Kebanyakan pesakit adalah berbangsa Melayu 76 orang (80.0%), tidak merokok 85 orang (89.5%), tanpa penyakit perubatan (86.4%), tiada rebakan jauh 54 orang (56.8%) dan tiada penglibatan kelenjar limfa 67 kes (70.5%). Umur min adalah 53.89. Faktor yg berkaitan dengan positif ekspresi ADAM9 secara statistic adalah saiz kanser (p=0.004), rebakan jauh (p=0.009) dan jenis histology bagi kanser iaitu karsinoma sel skuamus (p=0.017). Wanita yang menghidapi kanser sel skuamus, mempunyai kebarangkalian sebanyak 7.39 untuk mendapat ekspresi ADAM9 berbanding dengan wanita yang menghidapi adenokarsinoma (95% CI 1.42, 38.51; p=0.017). Pesakit kanser serviks yang ada rebakan jauh mempunyai peluang sebanyak 12.82 untuk mendapat ekspresi ADAM 9 berbanding dengan pesakit yang tiada rebakan jauh (95% CI 1.91, 86.13; p=0.009). Dengan setiap kenaikan tisu barah sebanyak 1mm, pesakit mempunyai 1.08 peluang untuk mendapat ekspresi ADAM 9 di dalam tisu barahnya(95% CI 1.02, 1.13; p=0.004).

Abstract

Cervical cancer is a preventable and the third most common cancer to occur in the reproductive age women. It is caused by the Human Papilloma Viruses. The ADAM 9 protein is involved in basement membrane degradation and tumor metastasis in certain types of tumor. Objective of this study is to describe the characteristics of cervical cancer and factors associated with ADAM 9 expression in cervical cancer. Retrospective record review was done in February 2012 using patients' case notes obtained from the Medical Record Unit, Universiti Sains Malaysia Hospital. Data was entered and analyze using SPSS version 19. There were 95 cases of cervical cancer included in this study. Majority were Malays 76 (80.0%), non smoker 85 (89.5%), without co-morbid disease (86.4%), had no distant metastasis 54 (56.8%) and absent of lymph nodes involvement 71 (74.7%). Squamous cell carcinoma was the most common type of cervical cancer 67 (70.5%). Mean age of subjects were 53.89 (10.83). The statistically significant associated factors for ADAM9 positive staining among cervical cancer were tumor size (p=0.004), distant metastasis (p=0.009) and histological type of cervical cancer, which is squamous cell carcinoma (p=0.017). Women with squamous cell carcinoma of cervical cancer has 7.39 times odds of having ADAM9 staining positive as compared with women with adenocarcinoma of cervical cancer (95% CI 1.42, 38.51; p=0.017). Cervical cancer patients who had distant metastasis has 12.82 times chances of having ADAM9 staining positive as compared with cervical cancer patients without distant metastasis (95% CI 1.91, 86.13; p=0.009). while with every 1mm increment in tumor size, women with cervical cancer will had 1.08 odds times od having ADAM9 staining positive (95% CI 1.02, 1.13; p=0.004).

1 INTRODUCTION

1.1 ANATOMY

The female reproductive organs composed of the vagina, uterus, fallopian tubes and the ovaries located within the pelvic cavity,

The ovaries are oval shaped organs normally measures two by four centimeters each and connected by the mesovarium to the broad ligament. The ovaries are situated at the right and left of the uterus and connected to the uterus by the round ligaments of the ovary. The ovaries usually lie in the ovarian fossa. The functions of the ovaries are to house the ova and also produced the sex hormones – i.e. the estrogen and progesterone.

The fallopian tubes are hollow organs attached to both sides of the uterus at the fundus part. Beneath them is the broad ligament. The uterine tubes are divided into four part – the infundibulum (funnel shaped part which it end formed finger-like structures call fimbriae), the ampulla (the widest part), the isthmus (the narrowest part located just lateral to the uterus) and the intramural part (the part which pierce into the uterine wall). The lumens of these fallopian tubes are continuous with the uterine cavity. The function of the fallopian tube is to receive the ovum and mobilized the fertilized ovum to the uterine cavity.

The uterus is a pear-shape, hollow and muscular organ and normally measures eight centimeter long, five centimeter wide and two centimeter thick. The uterus can be divided into three parts – i.e. the fundus (lies above the entrance of the uterine tubes), the body (lies below the entrance of the uterine tubes) and the cervix (the narrowest and distal-most part). Anteriorly, the uterus is related to the uterovesicle pouch and the superior surface of the external bladder. The cervix is related to the anterior fornix of the

vagina. Posteriorly, it is related to the pouch of Douglas and coils of ileum and sigmoid colon. Laterally, it is related to the broad ligament and the uterine artery and vein, the lateral fornix of the vagina and also the ureter.

The uterus is supplied mainly by the uterine artery which is a branch of the internal iliac artery. Small descending branch of the uterine artery also supplies the cervix and vagina. The veins follow similar pathways as the artery and drains into the internal iliac artery. The lymph vessels from the fundus of the uterus drains into the para-aortic nodes, and the lymph vessels from the body and the cervix drains into the internal and external iliac lymph nodes. A small number of the lymph vessels also follow the round ligament and drain into the superficial inguinal lymph nodes.

1.2 HISTOLOGY

Even though the muscular layer of these organs is the same, the lining of the uterus and the cervix is different. The lining of the cervix can be divided into two parts – the endocervix (the cervical canal) and the ectocervix (the cervical layering the cervical part which protrudes in to the vagina cavity). The endocervix is lined by simple columnar epithelium. These endocervical lining also secretes mucus. The ectocervix has a different kind of lining – the non-keratinizing stratified squamous epithelium. In between these two lining (the endo- and ectocervix), there is a transformation zone. Over this area there is a transition of lining from the simple columnar epithelium to the non-keratinizing stratified squamous epithelium. The lining in the transformation zone is prone to transform into cervical carcinoma.

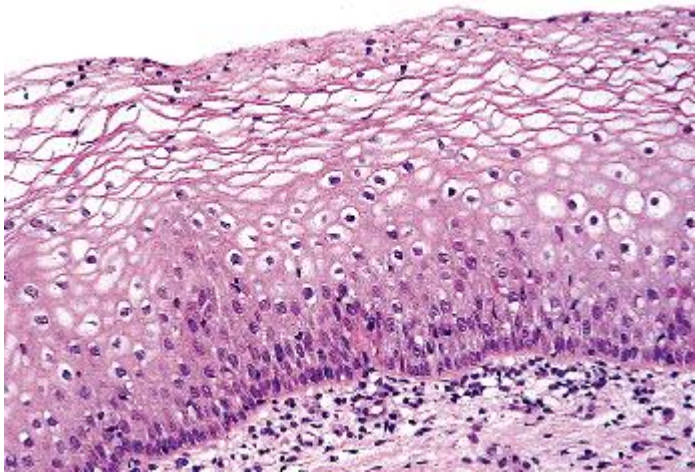

Figure 1: Histology of Ectocervix, Stratified squamous epithelium.

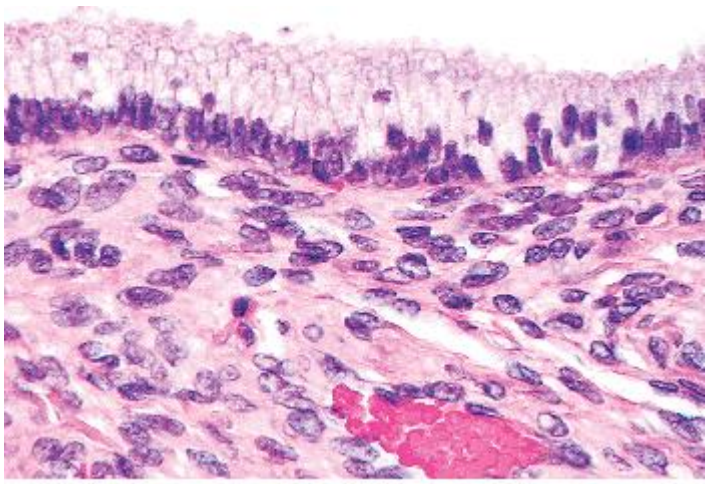

Figure 2: Histology of endocervix, Tall columnar epithelium.

1.3 PATHOLOGY AND PATHOPHYSIOLOGY

1.3.1 Pathology

World Health Organization (WHO) has divided cervical carcinoma into few subtypes. It is divided into epithelial tumor, the mesenchymal tumor, the mixed epithelial and mesenchymal tumor, melanocytic tumor, miscellaneous tumor, lymphoid and hematopoietic tumor and secondary tumor. WHO then further classify the epithelial tumor into few categories – i.e. squamous tumor and precursors, glandular tumor and precursors, and other epithelial tumors.

The squamous cell carcinoma is the most common type of the epithelilal cancer of the cervix, which accounts for 80% of total cervical cancer followed by the adenocarcinoma (15% of total cervical cancer) and adenosquamous and other types of cancer which only comprise about 5% of the cervical cancers. All of the common types of carcinoma of the cervix (squamous cell carcinoma, adenocarcinoma and adenosqamous carcinoma) are caused by high oncogenic Human Papilloma Viruses (HPVs).

The type of cervical cancer can be distinguished by evaluating the histology of the lesion under the microscope. There are few characteristic features that differentiate between the squamous cell carcinoma, the adenocarcinoma and the adenosquamous carcinoma of the cervix.

1.3.1.1 The squamous cell carcinoma of the cervix

Apart from the most common type of cancer to occur in the cervix, squamous cell carcinoma of the cervix is also the most common type of carcinoma to occur in women. However, the incidence of the cervical carcinoma has decreased in developing country and the credit goes to the intense screening program, i.e. the usage of the cervical Pap

smear and the increased in the awareness of the women on the importance of the Pap smear.

The squamous cell carcinoma of the cervix has a peak incidence in women in their fifth and sixth decades of life. But some cases do occur in a younger age group. Early marriage, early encounter of the first sexual intercourse, multiparity and low social-economic status are closely associated with squamous cell carcinoma of the cervix.

Human papilloma viruses (HPV) and squamous cell carcinoma of the cervix are well-known to be associated. The association between the cervical cancer and HPV was first discoverd by Harald zur Hausen in 2008. Since then, many cervical squamous cell carcinoma researches are based on the HPV. However, not all women infected with the HPV will develop squamous cell carcinoma. Only women who had recurrent and unresolved HPV infection will develop cervical cancer. The close relationship between the cervical cancer and the HPV infections show that cervical cancer is a preventable disease.

The squamous cell carcinoma of the cervix is composed of malignant squamous cells. Grossly the tumor is either exophytic (protruding mass from the cervix) or endophytic mass (invasive to the surrounding structures). These malignant squamous cells are different from the squamous cells that lined the ectocervix. The squamous cell carcinoma will show anastomosing bands of malignant cells within the stroma. This pattern of growth will formed irregular islands of malignant cells. Some of these “islands” are rounded but majority of it are spiked or angulated. There will be infiltrations by various types of inflammatory cells seen within the intervening stroma. There are many subtypes of squamous cell carcinoma of the cervix. The easiest way to classify this tumor is by dividing them into: 1) keratinizing and 2) non-keratinizing.

The keratinizing squamous cells carcinoma of the cervix shows formation of keratin pearl – whorls of squamous cell with keratin nest in the centre. Cytologically the nucleus is enlarged and hyperchromatic and has coarse chromatin. The mitosis; however, is infrequent unless in a less differentiated tumor. The non-keratinizing squamous cells carcinoma of the cervix composed of polygonal squamous cells that show individuals keratinization and intercellular bridges, however no keratin pearl can be seen. The mitosis is easily seen and the nuclear and cellular pleomorphism is more marked in this type of cervical carcinoma. Even though the non-keratinizing squamous cell carcinoma show a similar histological pattern as the keratinizing squamous cell carcinoma, the difference between these two tumor subtype lies in the formation of the keratin pearl. In the non-keratinizing type, there is no keratin pearl formation noted.

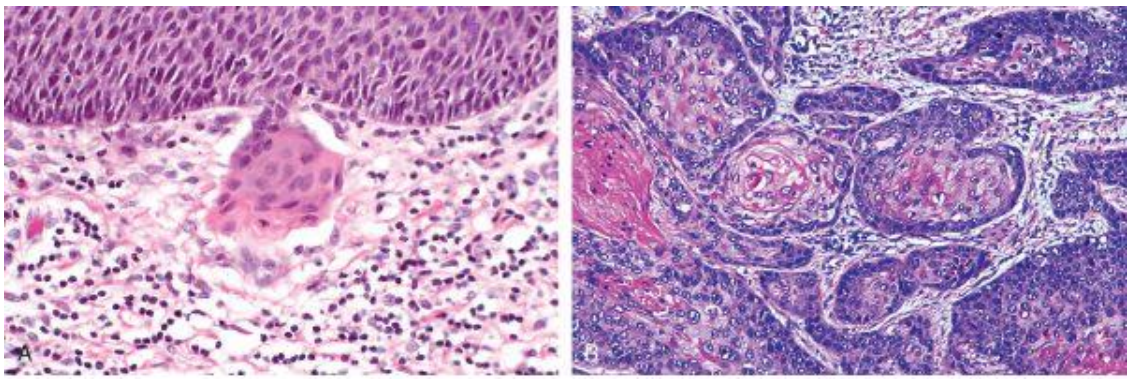

Figure 3: Squamous cell carcinoma. (Kumar, Abbas et al. 2010)

The squamous cell carcinoma spread through several routes. This cancer can spread to the vagina, the body of the uterus, parametrium, lower urinary tract and uterosacral ligaments via direct extension. The lymph nodes can also be involved by the tumor and these include the paracervical, hypogastric, obturator, external iliac, sacral, common iliac, aortic and inguinal groups. Bone and lung is also common site for distant metastasis. This tumor can be treated by surgery, radiotherapy or a combination of both.

The prognosis of the squamous cell carcinoma is related to few parameters – i.e. the clinical stage, lymph nodes involvement, site of lymph nodes involved, size of the primary tumor, the depth of invasion, microscopic grade, tumor associated tissue eosinophilia (TATE), cell proliferation index, angiogenesis and HPV (especially type 16 and 18) infection.

1.3.1.2 The adenocarcinoma of the cervix

Five to fifteen percent of cervical carcinoma consists of adenocarcinoma. The well differentiated glandular pattern is the most common type. This type of cancer is mucin producing tumor and some of the mucin can be spilled over into the stroma and this mucin show positivity with alcian-blue and mucincarmine staining. There are also few other variants and a poorly differentiated form of the cervical adenocarcinoma.

Morphologically, both endometrial and cervical adenocarcinomas look very similar. It is important to determine which part of the uterus the adenocarcinoma comes from. The points that support the adenocarcinoma is arising from the cervix rather than endometrium is the presence of the in situ component of the cervical glands, diffused staining of intracellular mucincarmine and CEA. They also have known to be negative for vimentin, estrogen receptor and progesterone receptor staining. In situ hybridization will proof that there is a presence of HPV.

A few morphologic variant can be seen in the adenocarcinoma of the cervix. The variants include endometrioid adenocarcinoma, papillary serous carcinoma, adenoma malignum, villoglandular (papillary) adenocarcinoma, adenosquamous (mixed) carcinoma, glassy clear cell carcinoma, adenoid basal cell carcinoma, clear cell carcinoma and mesonephric (adeno)carcinoma.

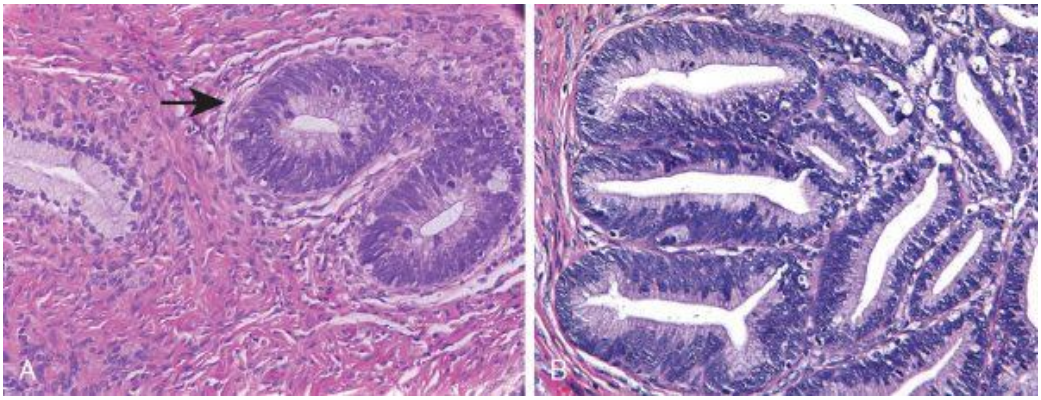

Figure 4: Adenocarcinoma of the cervix. (Kumar, Abbas et al. 2010)

The adenosquamous carcinoma is more alike the adenocarcinoma except, in this type, there is a presence of a squamous cell differentiation.

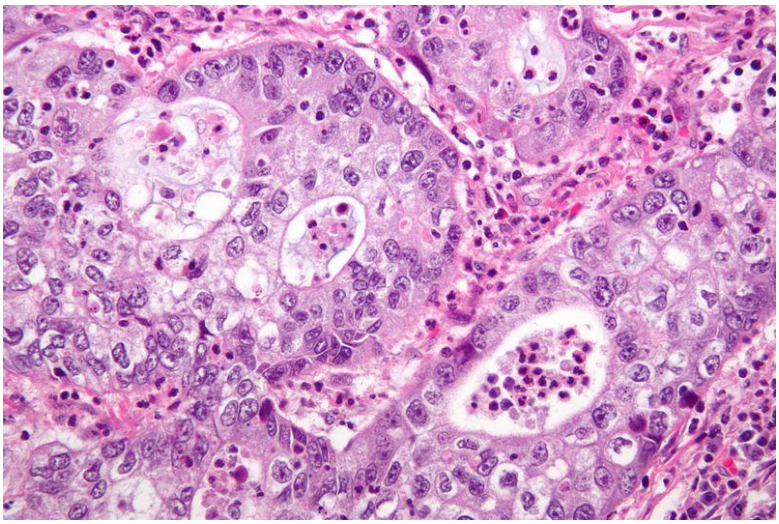

Figure 5: Adenosquamous carcinoma. (Kumar, Abbas et al. 2010)

1.3.2 Pathophysiology

The common types HPV which is associated with cervical cancer are (arranged in descending order of frequency) 16, 18, 33, 45, 31, 58, 52 and 35. Fifty-four percent of squamus cell carcinoma and 41% adenocarcinoma of the cervix are associated with HPV 16 infection. Whereas 11% squamus cell carcinoma and 37% adenocarcinoma of the cervix are associated with HPV 18 infection. These findings concluded that HPV 16 and

18 are the most common etiological agents for the squamus cell carcinoma and adenocarcinoma of the cervix. HPV will infect the basal cell layer with the help of the receptors (heparin sulphate proteoglycans and α6-integrin) available in the dividing basal cells. The viral particle is then integrated with the host (basal cells) DNA to replicate. The viral particles which are important for the development of the cervical cancer are E6 and E7 proteins. These proteins will inhibit apoptosis and prevent DNA damage repairs. Basal cells which expressed E6 protein will be inhibited from apoptosis, altered response to DNA damage and will have accumulated genomic mutations. Most of the infected women will resolved spontaneously, but in women who have repeated infection and re-infection and those who have unresolved infection will progress into high grade intraepithelial lesion and progress into cervical cancer (squamus cell carcinoma, adenocarcinoma or adenosquamus carcinoma) (Gonzalez Martin, 2007).

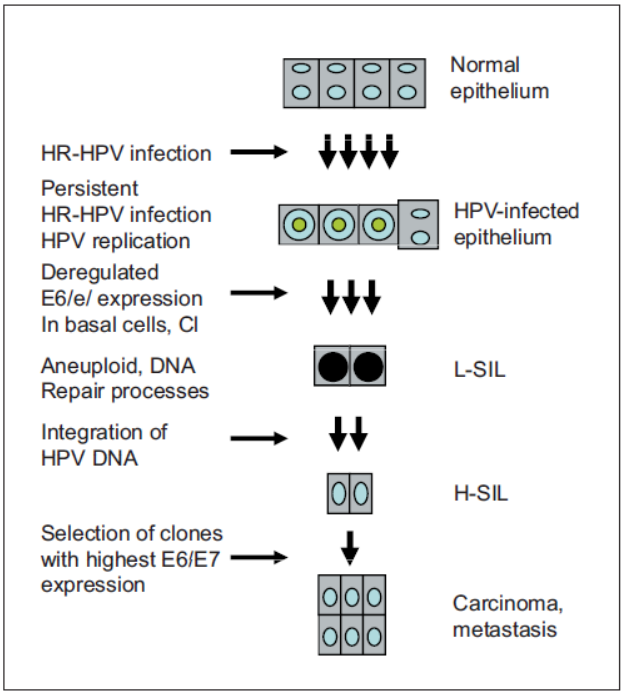

Figure 6: Pathogenesis of HPV related cervical cancer. (Boulet, Horvath et al. 2007)

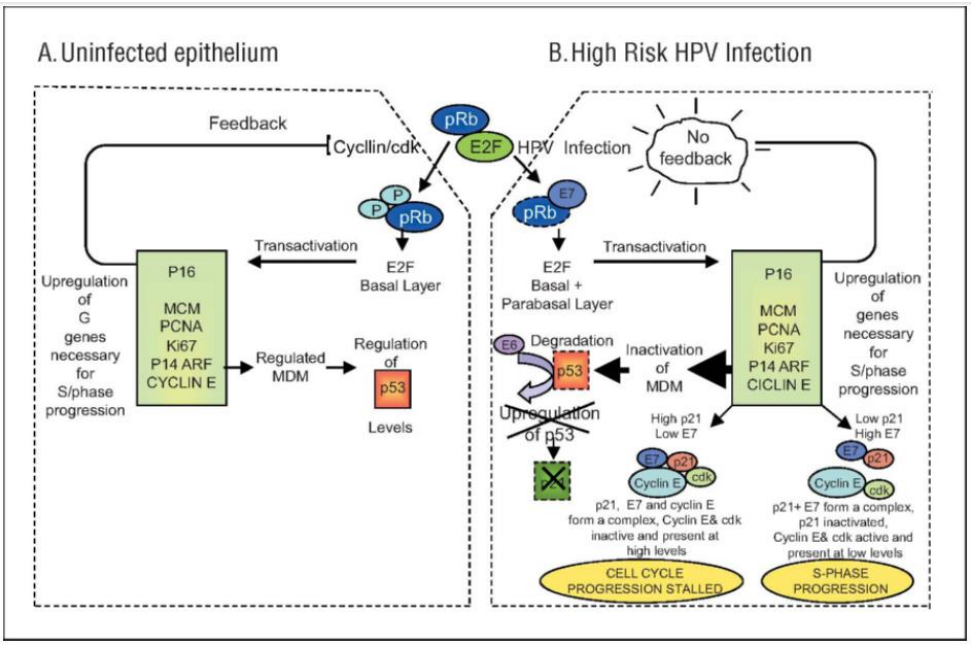

Figure 7: Pathogenesis at molecular level. (Boulet, Horvath et al. 2007)

1.4 EPIDEMIOLOGY

Cervical cancer is the second most common cancer for women worldwide. According to WHO, 500 00 new cases and 250 000 death due to cervical cancer occur in 2005. Most of the deaths (80%) occur in developing country and it is estimated to rise up to 25% in the next 10 years. Majority of the death occur in women who are in their reproductive age. Within this age, these women might be a care-taker of the family, or contributing to the social and economic growth of their country. Although the statistic data is worrying, cervical cancer is still preventable and treatable (either by surgical, radiotherapy, chemotherapy or combination therapy) (WHO, 2007).

In between 2003 to 2005, the third common cause of death in government hospital in Malaysia is malignancy. And from the national cancer registry in 2006, cervical cancer is the fourth common cancer among all of other type of cancer and among Malaysian women alone, cervical cancer is the third common cancer to occur. Most of the cervical cancer patients in Malaysia are within 30 to 59 years of age, i.e. the productive age group. In comparison of ethnicity (from three major ethnic group i.e. Malay, Chinese and Indian) cervical cancer incidence is high among Malay and Chinese lady (Malay-42%; Chinese-47.7%). Whereas in Indian lady the incidence is lower (10.3%). In 2006 alone, there is 1074 new cervical cancer cases reported. Majority of the cases are contributed by the women between the ages of 30 to 60 years of age (National Cancer Registry, 2006).

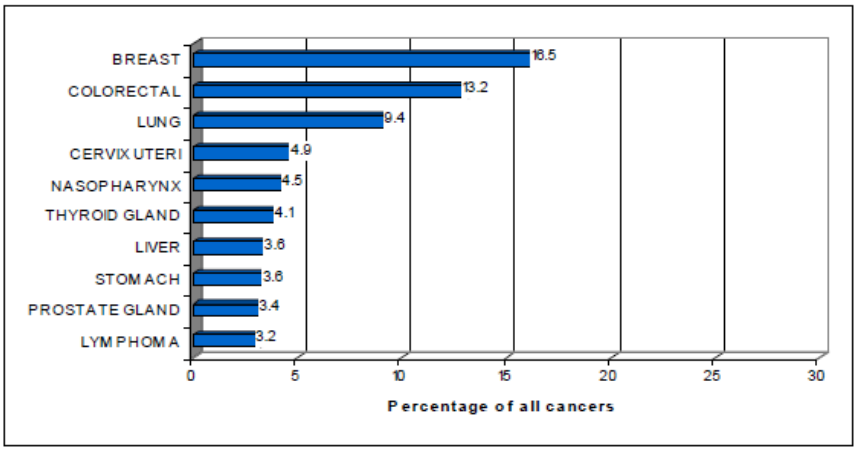

Figure 8: Ten most common cancer in Malaysia (National Cancer Registry 2006)

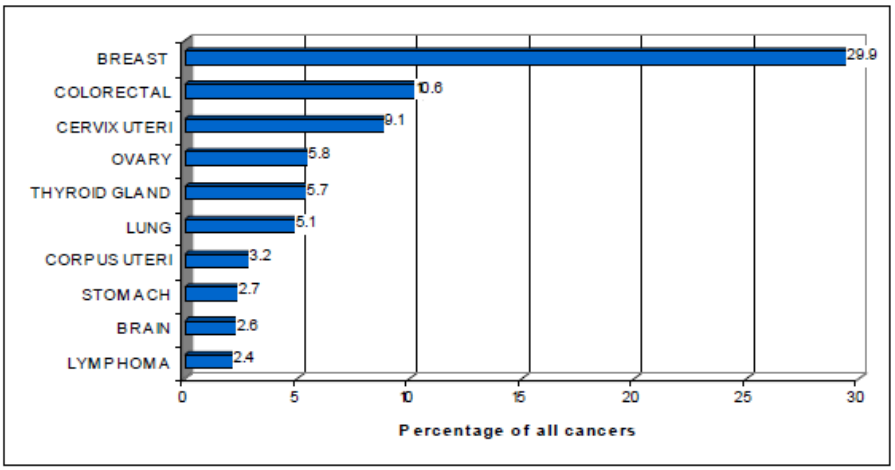

Figure 9: Ten most common cancer among women in Malaysia (National Cancer Registry 2006)

1.5 CLINICAL PRESENTATION

Previously the most common clinical presentation for cervical cancer is post coital bleeding. But due to the extensive cervical cancer screening (Pap smear) by the Malaysian government, the findings of squamus cell carcinoma of the cervix is usually trough the screening programs. Exception is for the adenocarcinoma and adenosquamous carcinoma of the cervical. The later carcinomas usually presented with advanced stage because adenocarcinoma and adenosqamous carcinoma of the cervix tend to have a false-negative result on Pap smear (Kumar et al., 2010, Tavassoli and Devilee, 2003, Pak et al., 2007).

1.6 A Dysintegrin and A Metalloproteinase

Malignant tissue has the ability to proliferate and progress (invade and metastasis). Similar abilities are also observed in cervical cancer. These abilities are caused by the mutation and production of various proteins and enzymes (Malur et al., 2001, Noguchi et al., 1987). One of the newly discovered group of proteins which involved in the tumor proliferation and progression is the A disintegrin and metelloproteinases (or ADAM). These proteins share similar sequence as in the reprolysin found in the snake venomases. The matrix metalloproteinases also shared the metalloproteinases domain as in the snake venomases. The ADAM proteins involved in various biological functions such as cell adhesion, fusion and migration, membrane protein shedding and also proteolysis. Many ADAM proteins are expressed in malignant tumor. These proteins are involved in growth factor activities and integrin functions. The sum of these is the promotion of tumor cells growth and invasion (Mochizuki and Okada, 2007, Murphy, 2008, Zigrino et al., 2002).

2 LITERATURE REVIEW

2.1 Cervical cancer

2.1.1 Anatomy and Pathogenesis

Cervix is a part of a female genital organ known as uterus. It is located at the distal-most part of the uterus. The cervix is demarcated by internal and external os. The external os is the opening of the cervical canal into the vagina and the internal os is the opening of the cervical canal into the uterine cavity. Cervix is mainly lined by two type of lining, i.e. the ciliated columnar epithelium at the cervical canal and the non-keratinizing stratified squamous epithelium externally. In between these two lining there is a transitional zone where there is a transformation of the epithelial lining from the squamous epithelium to the columnar epithelium. Located in the pelvic, it is related to several other pelvic organ including the vagina, ovaries, fallopian tubes, rectum and also urinary bladder. Cervix received similar blood supply as uterus. The blood supply is mainly from the uterine artery which is a branch of the internal iliac artery. The small descending branch of the uterine artery also supplies the cervix and vagina. The veins follow similar pathways as the artery and drains into the internal iliac artery. The lymph vessels from the fundus of the uterus drains into the para-aortic nodes, and the lymph vessels from the body and the cervix drains into the internal and external iliac lymph nodes. Few of the lymph vessels also follow the round ligament and drain into the superficial inguinal lymph nodes (Snell 2007). Diagrams below illustrate the female's pelvic cavity and organs (**John T. Hansen** 2005).

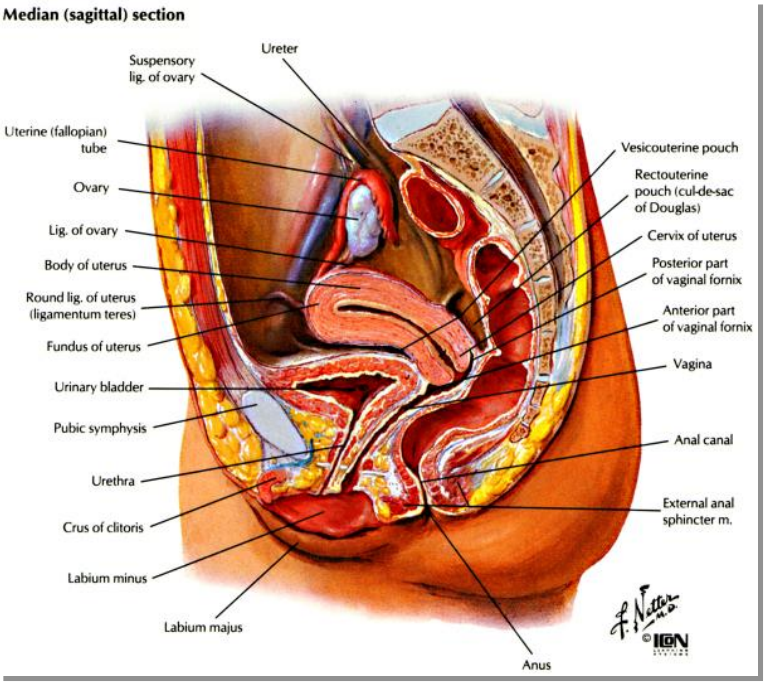

Figure 10: Saggital sections of female genital tract.

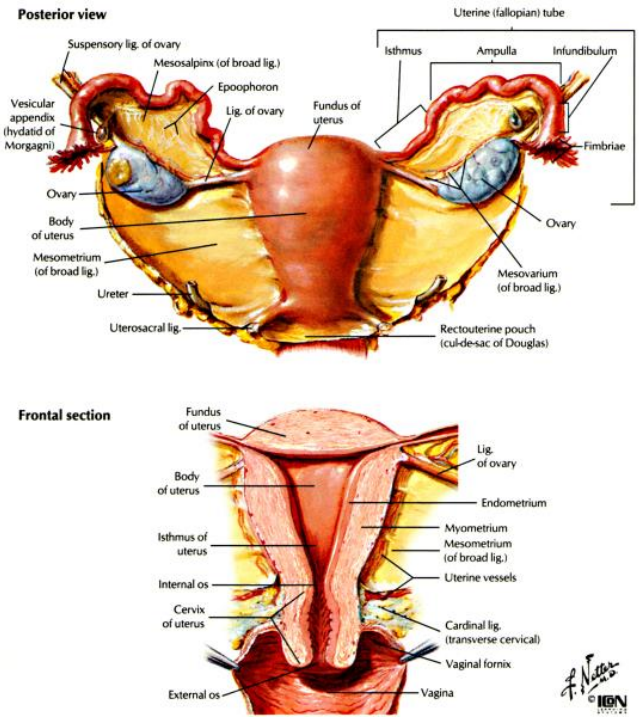

Figure 11: Intact and opened uterus

There are few causes or etiological factor to develop cervical cancer. The etiological factors for cervical cancer development are (Kumar, Abbas et al. 2010):

1) Cervical cancer is seen occurred in women who had previous history of sexual intercourse. The risk will increase if the women have multiple sexual partners or if the women had a sexual partner who practices prosmicuity. Cervical cancer is rarely encounters in nun, who is abstinence from sexual relationship. Due to it nature, cervical cancer is also to be considered as Acquired Immunodeficiency Syndrome (AIDS) defining disease (Kumar, Abbas et al. 2010).
2) Women who had been exposed to sexual intercourse at an early age and who had an early first pregnancy also had increase risk to develop cervical cancer (Louie, Sanjose et al. 2009).
3) High number of parity is also a risk to develop cervical cancer in some women (Kumar, Abbas et al. 2010).
4) Human papilloma virus (HPV) is a virus which cause genital warts. But in recent years the role of HPV in cervical cancer development is identified. Recurrent and unresolved HPV infection to the women genital tract is a main cause for cervical cancer development. There are more than 70 HPV subtypes. Only few types of this virus that cause cervical cancer and this will be discussed later (Gonzalez Martin 2007).
5) Women who had been exposed to nicotine are at risk to developed cervical cancer (Itzel, Calleja-Macia et al. 2009).
6) In women with low immunity, e.g. in women with diabetes mellitus or in AIDS, the incidence of cervical cancer is increased in compare to women who is healthy (Gallagher, Novosyadlyy et al. 2010).

Genital Human Papilloma Virus (HPV) infection is a common infection and many women who had been infected by HPV usually resolved spontaneously. There will be changes in the cervical tissue once a woman is infected by Human papilloma virus. Initially there will be some epithelial lining changes – cervical intraepithelial neoplasm (CIN). The CIN occur as a spectrum. It started with CIN I, then progress to CIN II and lastly transformed into CIN III. The difference of these three CIN is in the grade of dysplasia exhibits by them. CIN I has mild epithelial dysplasia, CIN II has moderate epithelial dysplasia and CIN III has severe epithelial dysplasia. These CINs are a spectrum, CIN I lesion can progress to CIN II and further to CIN III. CIN III is considered as malignant precursor as this lesion cannot regress back to CIN II or CIN I. CIN III will soon progress to cervical cancer. For the current classifications, the intraepithelial neoplasias are simplified into two-tiered classification – i.e. the low-grade squamous intraepithelial lesion or LSIL (previously CIN I) and the high-grade squamous intraepithelial lesion or HSIL (previously CIN II and CIN III). Sixty percent of low-grade squamous intraepithelial lesion will regress, 30% will persist and another 10% will progress into high-grade squamous intraepithelial lesion. Whereas 30% of the high-grade squamous intraepithelial lesion will regress to normal, 60% will persist and another 10% will progress into cervical carcinoma. Both the progression of LSIL into HSIL and from HSIL into cervical carcinoma will take approximately 2 to 10 years (Kumar, Abbas et al. 2010).

Each normal cell is prone to developed genetic mutations. These mutations will transform a normal cell into a neoplastic cell. But, human cells are unique. It has a capability to destroy and repair the genetic mutation. Two important genes that involved in the correction of the mutated cells are the *p53* gene and the *Retinoblastoma (RB)* gene.

These two genes are important in the pathogenesis of the cervical cancer in HPV's infection. More than 70 types of Human Papilloma Viruses have been identified. These human Papilloma Viruses can be divided into low-risk and high-risk group. For example, type 1, 2 and 4 belong to low risk group will usually caused benign squamous papilloma. Meanwhile, type 16 and 18 which belong to the high risk group had been identified to be associated with cervical cancer.

Human papilloma virus type 16 and 18 had specific proteins which facilitate in the pathogenesis of cervical cancer. The two proteins involved are the Human papilloma virus (HPV) E6 protein and HPV E7 protein. Both HPV E6 and HPV E7 proteins will enhance the *p53* degradation. The *p53* is an important gene that induced apoptosis in a genetically abnormal cell. When there is a genetic defect which is unable to be repaired, it will be detected by the repair protein and then this information is then conveyed to the *p53* gene to initiate apoptosis. In the presence of HPV E6 and HPV E7 proteins, the *p53* gene will be degraded and the whole process is inhibited. Apart from causing degradation of the *p53* gene, the HPV E7 protein will also bind to *RB* gene and causing uncontrolled growth in dividing cells. *Retinoblastoma* gene is important in mitosis. The function is to stopped any genetically abnormal cells from continue to undergo mitosis and divide. The mutated cells will have unopposed mitosis and will not undergoes apoptosis(Gonzalez Martin 2007). The inhibition of the apoptosis and also uncontrolled growth is "the tip of the iceberg" for the cancer cells mutation. Due to inability to correct the mutations (as a result of uncontrolled cell growth), the cancerous cells will have a lot of mutations. The mutations involved in cancer cells will determine the cancer cells survival, metastasis and invasion (Kumar, Abbas et al. 2010).

2.1.2 Pathology

There are few types of cervical cancer. According to the World Health Organization (WHO) classifications, the cervical cancer are divided into epithelial tumors, mesenchymal tumors and tumor-like conditions, mixed epithelial and mesenchymal tumors, melanocytic tumors, miscellaneous tumors, lymphoid and hematopoietic tumors and secondary tumors (Tavassoli and Devilee 2003). High risk Human Papilloma Viruses is proven to be the etiological factor for the epithelial tumors of the cervix, particularly the squamous cell carcinoma, adenocarcinoma and also adenosquamous carcinoma (Tavassoli and Devilee 2003).

Grossly, the squamous cells carcinoma of the cervix is a polypoid or deeply infiltrated tumor. The carcinoma (cancer) is being seen growing out from the cervical tissue or the cervix can be bulky in cases of deeply infiltrated squamous cells carcinoma. Microscopically, squamous cells carcinoma is formed by cancer cells with squamous differentiation – i.e. polygonal cells, abundant pink cytoplasm, pleomorphic and hyperchromatic nuclei, abundant cytoplasm and produced keratin. The later feature is evidence by the formation of keratin pearl and individual cells keratinisation. Despite the gross feature, this malignant cell is seen arising from the squamous lining and infiltrating the underlying structure – i.e. the muscular layer and the surrounding structure. Apart from the main feature, there are few variations of squamous cells carcinoma, particularly the large cell non-keratinising type, small cell type, verrucous carcinoma, spindle cell carcinoma and basaloid (squamous cell) carcinoma(Rosai 2004).

In adenocarcinoma of the cervix, grossly it lack “dramatic” feature as compared to the squamous cells carcinoma. The adenocarcinoma rises from the endocervix, where the lining is ciliated columnar and there is presence of the cervical glands there. Microscopically, the well-differentiated glandular carcinoma is seen infiltrating the

underlying layer of the cervix – i.e. the muscular layer and surrounding structure. The adenocarcinoma cells have hyperchromatic and pleomorphic nuclei with mucinous cytoplasm. Some of these cells also produced abundant amount of mucin. Other variants of adenocarcinoma includes the adenosquamous (mixed) carcinoma, endometrioid adenocarcinoma, serous (papillary) carcinoma, adenoma malignum, glassy cell carcinoma and villoglandular (papillary) carcinoma. Adenosquamous carcinoma of the cervix has more similar feature as in adenocarcinoma of the cervix. But, apart from the glandular pattern, squamous differentiations are also seen in this carcinoma (Rosai 2004).

Regardless the type of cervical cancer, in general they spread by direct extension. It usually spread to the cervix, parametrium, lower urinary tract and uterosacral ligaments. In advance disease, this carcinoma can spread to the lymph nodes and also elicits distant metastasis. In term of lymph nodes involvements, the paracervical, hypogastric or obturator groups are among the lymph nodes to be involved in earlier stage. The external iliac, sacral, common iliac, aortic or inguinal groups will be affected later (Rosai 2004).

The carcinoma of the cervix is staged using FIGO classification. This classification is simplified by using the table below (Tavassoli and Devilee 2003; Lester 2010):

Stage	Description
I	Cervical carcinoma confined to the uterus
IA	Invasive carcinoma by microscopy. IA1 – stromal invasion less than 3.0mm deep and less than 7.0mm horizontal spread. IA2 – stromal invasion in between 3.0mm to 5.0mm with horizontal spread of less than 7.0mm.

IB	Macroscopically visible lesion or microscopically more than IA2. IIB1 – lesion less than 4.0cm in greatest dimension. IIB2 – lesion more than 4.0cm in greatest dimension.
II	Tumor invasion is beyond the uterus but not invades the pelvic wall or the lower third of the vagina. IIA – invasion without parametrial invasion. IIB – invasion with parametrial invasion.
III	Tumor extends to the pelvic wall, involves the lower third of the vagina or causes hydronephrosis or non-function of the kidney. IIIA – tumor involves lower third vagina without entension to the pelvic wall. IIIB – tumor invades the pelvic wall or causes hydronephrosis or non-function kidney.
IV	IVA – tumor invades mucosa of bladder or rectum or extends beyond true pelvis. IVB – distant metastasis.

Table 1: Stage of cervical cancer (Tavassoli and Devilee 2003; Lester 2010)

2.1.3 Clinical

Early cervical carcinoma normally does not produce any symptoms. It is usually an incidental finding in autopsy or during histopathological examinationof the uterus removed for some other reasons. As the tumor grows, patients normally presented with vaginal bleeding (post-coital bleeding is the common presentation) and discharge. Extension of the tumor to the parametrium and invasion to the ureter will cause anuria or hematuria. Uremic symptoms are caused by non-functioning kidney invaded by the tumour. Some patients do complaint of sciatic or leg pain. This symptom is caused by the

extension of the tumor to the pelvic wall and compressing the nerve supplying to the lower limbs. Patient with anterior extension of the tumor will present with complaints of urinary frequency (Tavassoli and Devilee 2003; Rosai 2004). On speculum examinations, the tumor is a red, friable, exophytic or ulcerated lesion. In some cases nodularity at the parametrium can be felt via per-rectal examination (Tavassoli and Devilee 2003).

2.1.4 Epidemiology

Cervical cancer is the second most common cancer for women worldwide. According to WHO, 500 00 new cases and 250 000 death due to cervical cancer occured in 2005. Most of the deaths (80%) occured in the developing countries and it was estimated to rise up to 25% in the next 10 years. Majority of the death occur in women who are in their reproductive age. Within this age, these women might be a care-taker of the family, or contributing to the social and economic growth of their country. Although the statistic data is worrying, cervical cancer is still preventable and treatable either by surgical, radiotherapy, chemotherapy or combination therapy (WHO 2007).

In between 2003 to 2005, death due to malignancy was the third common cause of death in government hospital in Malaysiaaccording to the 2006 national cancer registry cervical cancer is the fourth most common cancer among all of other type of cancer and among Malaysian women alone, cervical cancer is the third common cancer to occur. Most of the cervical cancer patient in Malaysia is within 30 to 59 years of age, i.e. the productive age group. In comparison of ethnicity cervical cancer incidence is high among the Chinese and Malays (Chinese-47.7%; Malay-42% respectively). Whereas in Indian lady the incidence is lower (10.3%). In 2006 alone, there was 1074 new cervical cancer cases reported. Majority of the cases were contributed by the women between the ages of 30 to 60 years of age (National Cancer Registry 2006).

In a review done by world health organization in 2007, cervical cancer was common in women among the reproductive age group – i.e. 15 to 44 year-old group. Whereas, death due to cervical cancer was common in older women – i.e. 50 to 60 year-old women (WHO 2007).

In Malaysia respectively, there is increasing incidence of cervical cancer among Malaysian women after the age of 30 year-old. Among Malaysian women affected by the cervical cancer, peak incidence was at the age of 60 to 69 year-old. According to race, cervical cancer is commonly affected Chinese (47.7%), followed by the Malays (42%) and Indian (10.3%) (National Cancer Registry 2006).

2.2 ADAM 9

ADAM9 is an abbreviation for A-dysintegrin and A-metalloproteinase 9 protein. There are many ADAM proteins and currently there are 34 recognized ADAM proteins. ADAM proteins can be divided into two subtypes – the membrane anchored type (the ADAM) and the secretory type (the ADAMTS) (Mochizuki and Okada 2007). The ADAM proteins elicit proteinase activity (proteolytic). They involved both in biological as well as in pathological conditions. Some of biological functions that involved the function of ADAM proteins are ovulation, implantation of the fertilized ovum, menstruation, embryogenesis, uterus involution, bone resorption, vasculogenesis and hematopoeisis. The pathological conditions that involved ADAM proteins are tissue destruction and repair, inflammation, granulation, immune reactions, infection and angiogenesis. In neoplastic conditions ADAM proteins was found to play vital role for cancer cells proliferation and invasion (Takayuki Shiomi 2010). In breast, pancreas,

stomach, skin, liver and lungs cancers, the ADAM 9 protein involved in the cancer cells adhesion and invasion (Mochizuki and Okada 2007).

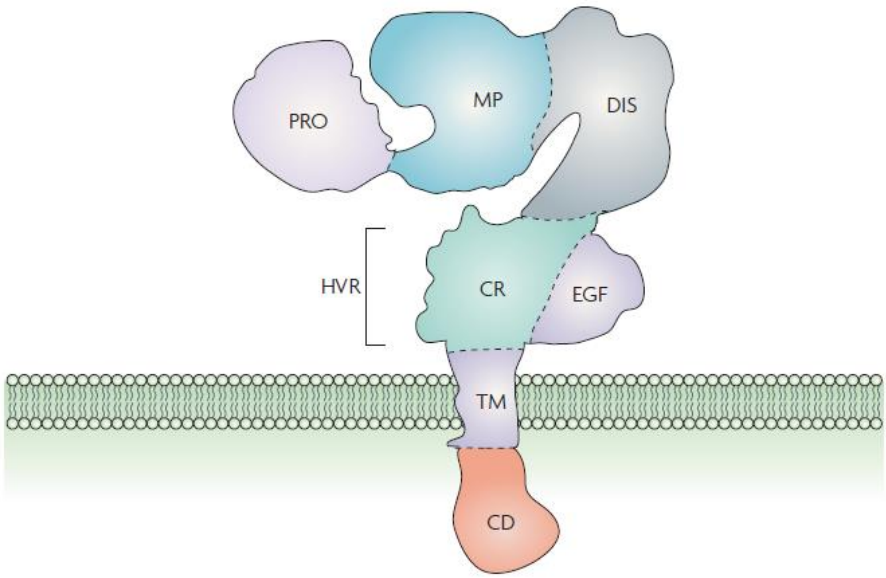

Figure 12: ADAM9 domain (Murphy 2008)

In one study done on hepatocellular carcinoma and colon cancer, ADAM 9 expression is seen increased in expression. The highly ADAM 9 expressed cancer is associated with more aggressive phenotype (Mazzocca, Coppari et al. 2005). ADAM 9 was also found to be highly expressed in renal cell carcinoma. It is also associated with high grade tumor and distance metastasis of the renal cell carcinoma (Fritzsche, Wassermann et al. 2008). In poorly differentiated adenocarcinoma of the pancreas, ADAM 9 protein is strongly expressed. Overexpression of the ADAM 9 protein was also observed in pancreatic ductal adenocarcinoma with poor prognosis (Grutzmann, Luttges et al. 2004). Non-small cell carcinoma of the lungs which metastatic to the brain was found out to be over-expressed of ADAM 9 protein (Shintani, Higashiyama et al. 2004).

ADAM 9 also can be used in targeted therapy. In research done in prostatic cancer patient, those who had ADAM 9 expression benefit more from the targeted therapy