Biospectra : Vol. 12(2), September, 2017, pp.97-104
An International Biannual Refereed Journal of Life Sciences

26(AS).
Animal Science

ISSN : 0973-7057

Metabolites and diagnosis of cervical cancer

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Received : 29th July, 2017; Revised : 30th August, 2017

Abstract : Cervical cancer (CC) is one of the most common types of gynecological malignancies worldwide that is particularly prevalent in the developing countries. Cervical cancer happens when cells change in women's cervix, which connects thier uterus with vagina. This cancer can affect the deeper tissues of their cervix and may spread to other parts of their body (metastasize), often the lungs, liver, bladder, vagina, and rectum. Prevention of cervical cancer very much relates to the prognostic and predictive biomarkers. To save many lives these markers are very important, as they help in taking clinical decisions at earliest. In this paper a comprehensive analysis of metabolite profiling technologies is performed.Metabolite profiling may allow us to extend diagnostic tests of cancer, and metabolite derivatives can be used for the molecular imaging of cancer.

Keywords : Cervical cancer, Metabolites, diagnosis, derivatives

INTRODUCTION

Epidemiology of cervical cancer

All over world, majority of deaths are caused by non communicable diseases.[1] In India, the prevalence of cancer is estimated to be around 2.5 million, with about 8, 00,000 new cases and 5, 50,000 deaths per annum.[2] According to World Health Organization, over 13.1 million deaths from cancer are projected in 2030 Worldwide. Developing countries contribute more than 25% global burden of cervical cancer. Among all types of cancers, cervical cancer holds rank four for occurrence and death related to cancer in women. In India cervical cancer accounts for round about 14% of all female cancer cases and about 17% of all cancer deaths among women aged between 30 and 69 years. It is estimated that cervical cancer occurs in roughly 1 in 53 Indian women during their lifetime.[3]

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Cervical cancer is a malignant neoplasm that begins with cells derived from cervix squamocolumner junction of the uterine cervix. A continual infection with human papillomavirus (HPV) is major cause of cervical cancer. HPV infection is a very common sexually transmitted disease that is also able to cause many other cancers like, anus, vulva, oropharyngeal, penile cancer.[4,5] Anogenital warts, recurrent respiratory papillomatosis and some head and neck cancer pathology is also found to be associated with HPV infection. Although infection with oncogenic human papillomavirus is essential for cervical cancer development, it alone is not enough to explain the development of cervical cancer. Therefore, other risk factors such as genetic factors of host, age, poor hygiene, smoking[6], sexual activity at a young age, several sex partners, oral contraceptive drugs[7], high number of pregnancies, and other sexually transmitted infections like co-infection with *Chlamydia trachomatis* (CT)[8,9] and

herpes simplex virus type-2 (HSV-2) certain dietary deficiencies and immunodeficiency[10] are also may be important in cancer progression.[11,12]

Human Papilloma Virus structure and its role in cervical cancer progression

Based on strong epidemiological evidence, supported by basic experimental findings, there is no doubt that persistent infections with high-risk types of human papillomavirus (HPV) represent a necessary cause of cervical cancer.[12] It is the single most important etiological agent in cervical cancer, contributing to neoplastic progression through the action of viral oncoproteins, mainly E6 and E7[13]. More than 100 subtypes of HPV are found, in which 13 have been identified as high risk strain (high-risk HPV) and causal reason for cervical neoplasias and other anogenital and oropharyngeal cancers.[12] According to the World Health Organization more than 78% cervical cancer cases are caused by HPV-16 and -18 and other strains.HPV-31, -33, -35, -39, -45, -51, -52, -56, -58 and -59 are also defined as high-risk HPV types.[14] Generally HPV infections become clear in a time period of 6-18 months and if it persists it will lead to progression of cervical intraepithelial neoplasia (CIN).[15,16] Early recognition of anomalies in cervical cells and HPV infection must be detected as soon as possible to prevent cervical cancer and reduce the number of deaths related. HPV spread primarily by skin-to-skin contact. HPV after entering in the host infects the basal cells, positioned between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix.

Human papillomavirus is a nonenveloped, small, epitheliotropic double-stranded DNA virus. Its genome exists in icosahedral capsid shell, which is formed of 360 copies of the L1 gene product and smaller L2 proteins that helps in linking the capsid to its DNA contents, are also present. This genome is separated into three regions: an early region (E), late region (L), and a LCR (long control region). Core proteins (E1, E2, L1 and L2) and accessory proteins (E4, E5, E6 and E7) are the gene products of HPV. Core proteins directly take part in viral genome rep-lication (E1 and E2) and virus assembly (L1 and L2), and are highly conserved among papilloma viruses (E-early, L-late, when transcription occurs in viral life cycle). Accessory proteins display change ability in their functional features and timing of expression.[17] Genes that encode accessory proteins modify the infected cell to ease virus replication.[18] Between E6 and L1 a non coding upstream regulatory region LCR is found that contains a core promoter sequence, enhancer and silencer sequences, needed for viral replication and transcription.[19] E6 and E7 enhance fitness of HPV, virion production and helps to amplify genome in differentiating epithelial layers.[20] L2 protein cause the production of neutralizing antibodies against different types of HPV and thus L2 protein is considered essential for potential vaccines development. HPV transform infected cells to cancer cells via a very complex process. It exploits the cellular machinery. In epithelial cell differentiation cycle, viral replication plays a significant role. Pro-mitotic proteins encoded by early genes help in the expression of viral replication factors. Terminal cell differentiation leads to activation of late gene expression and virions are produced on epithelium. When basal cell DNA replication is done, the HPV DNA replication begins. E1 and E2 are mandatory for the persistence of viral genomes in the host cells as they are crucial sites for viral DNA replication and for replication they utilize cellular DNA polymerase. Because of Oncoproteins E6 and E7, Cellular proliferation occurs; infected cells and infectious virions are increased as a result. A normal cell is now converted into cervical cancer cell and further epithial tissue of cervix progresses towards CIN (cervical intraepithelial neoplasia, CIN 1, CIN 2, and CIN 3), that develops into invasive cervical cancer.[19, 21]

Cervical cancer diagnosis and prevention strategies

Cervical cancer diagnosis has an important role and it aims to identify precancers and early cancers those are treatable so that invasive cancers can be prevented and the reduction in overtreatment is possible, in that way cancer mortality and treatment-related morbidity can be reduced. Understanding for the determination of investigative markers of cervical cancer is relatively little and it is not easy to detect the disease early. For more than 50 years the Pap smear has been the mainstay of cervical screening resulting in a dramatic decrease in death from cervical cancer. However, the Pap smear has certain disadvantages. It has a low sensitivity and high false negative rate.[22,23] However the sensitivity of the test is 60-80%, specificity is 70-95%, false negative rate is 15-50% and false positive is 30%. Other methods of cervical cancer screening include cytology, the detection of DNA or RNA of high-risk HPV types; and cytology–HPV co testing.

Most widely used screening is done with the visual inspection of cervical surface with acetic acid (VIA). Some clinical trials suggested that VIA screening is able to reduce cervical cancer mortality by 30%, and some systemic studies have shown limited sensitivity. HPV testing is much sensitive and specific if compared to VIA.[24,25] In addition magnified VIA (VIAM) visual inspection with Lugol's iodine (VILI), the Papanicolaou test are also some other screening methods in use. When compared with cytology-based screening, HPV-based screening resulted in a 60–70% decrease in invasive cervical cancer prevalence.[26] After the diagnosis of pre-cancer, colposcopic assessment is needed with cervical biopsies. p16/Ki-67 cytology has higher sensitivity at comparable specificity to cytology.[27] Other molecular markers such as host methylation and HPV methylation are also being investigated.[28]

Histopathology is also an authentic option to decide how women having HPV-related precancer and cancer should be treated. Cryopen and cold coagulation are some easy ablative methods with promising results.[29] Since HPV is the major risk factor in cervical cancer and after finding the relation between HPV and cervical cancer, primary and secondary prevention strategies came into the light. Primary prevention is done with the help of HPV vaccination and secondary prevention through HPV assays to discover precancers and early cancers. Vaccination is the most efficient intervention in the control of high-risk HPV infections and so in HPV-related cancers. Many countries have not yet initiated major vaccination programmes and there is a high rate of cervical cancer prevalence. In some other countries vaccination is unable to help old age females and thus secondary prevention is the most significant strategy in prevention.

Importance of Metabolic changes in cancer diagnosis

Cancer metabolism very much differs from normal cell metabolism. This difference in metabolism can be used as biomarker to recognize the onset and progression of cancer.[30] Tumour metabolism is modified by interaction with other cells in the microenvironment, tumour hypoxia, nutrient limitation and by tissue-specific signalling. Several therapies target multiple metabolic processes, significant for tumour growth and survival, including metabolism of amino acid, nucleotide, carbon and fatty acid. Alterations to cellular metabolism should be considered a crucial hallmark of cancer. Thus better understanding of metabolic reprogramming may lead to the identification of important control points that will help in diagnosis or that will be specific targets for the control of the disease. Metabolic phenotypes can also be exploited to image tumours, provide prognostic information, and treat cancers. Therefore, understanding cancer metabolism has implications for understanding basic cancer pathophysiology and for clinical oncology.

First key discovery on cancer cell metabolism was made by Nobel laureate Otto Heinrich Warburg, known as Warburg effect. It explains that cancer cells tend to favour metabolism via aerobic glycolysis rather than the much more efficient oxidative phosphorylation pathway, which is the preference of most other cells of the body. He demonstrated that aerobic glycolysis is an advantage for tumour cells.[31] Cancer cells are dependent on increased glucose uptake and it is useful for tumour detection and monitoring. This phenotype acts as the basis for clinical [18F] fluorodeoxy glucose positron emission tomography (FDG–PET) imaging.[32] Clinical data also suggest that glucose is important as a fuel for malignancies.

Several regulatory pathways participate in the metabolic reprogramming of tumour cells. The PI3K pathway is a common altered signalling pathway in human cancers, activated by mutations in tumour suppressor genes, such as PTEN. Mutations in the components of the PI3K complex itself or by aberrant signalling from receptor tyrosine kinases22 exerts effects on tumour cell metabolism. AKT1 drives the tumour glycolytic phenotype and also stimulates generation of ATP, so that cells can give response to growth signals[33], AKT1 stimulates glycolysis by enhanced expression of hexokinase and phosphofructokinase 2 and increased protein glyco sylation.[34] Activated mTOR stimulates protein and lipid biosynthesis and cell growth in response to sufficient nutrient and energy conditions and is often remain constitutively activated during Tumorigenesis.[35] A number of oncogenic mutations and signalling pathways can suppress AMPK signalling. AMPK acts against the effects of AKT1 and works as a inhibitor of mTOR and help in inhibition of cell proliferation,[36] numerous cancer cells show evidence of a loss in appropriate AMPK signalling.

Various other transcription factors also participate in the metabolic reprogramming of tumour cells. Major transcription factors, HIF1 and HIF2 (hypoxia-inducible transcription factor) are responsible for gene expression changes when the cell is in low oxygen conditions. HIF1

activates the glycolysis and pyruvate dehydrogenase kinases (PDKs). HIF also stimulates angiogenesis by upregulation of vascular endothelial growth factor (VEGF) and some other factors. The oncogenic transcription factor Myc also significantly affects metabolism of cell.[26] Myc activates glucose transporters in collaboration with HIF.[37] It increases metabolism of glutamine also.[27] Nuclear transcription factor p53 is another main regulator of metabolism.[28] Wild-type p53 helps and supports the expression of PTEN, in the inhibition of PI3K pathway. The expression of transcription factor OCT1 is also found increased in numerous human cancers, and it may help p53 in regulating the balance between glycolytic and oxidative metabolism.[38] For the energy of cell metabolism, balance is required together with macromolecular building blocks and maintenance of redox balance.

Many tumour cells express PKM2 that is an isoenzyme of the glycolytic enzyme pyruvate kinase. It has documented as an advantage to cancer in various studies.[39] Additionally NADPH is very important for biosynthesis of macromolecules, and also acts as a crucial antioxidant, for quenching of reactive oxygen species (ROS), produced all through rapid cell proliferation. Isocitrate dehydrogenases, IDH1 and IDH2 are enzymes that act in the mitochondria and cytoplasm to produce NADPH. Mutations in IDH1 and IDH2 are related to tumorigenesis.[40]In proliferative cancer cells, ROS regulation is crucial in abnormal metabolism and protein translation. GSH and Trx molecules help to reduce disproportionate levels of ROS to prevent irreparable cellular damage.[41]

If a cancer cell has lost the function of the tumour suppressor retinoblastoma (rb), and ROS production is also occurring, cell will go for apoptosis and oxidative stress will also increase. Many tumour cells are crucially dependent on glutamine amino acid and it is found in some studies that transformation stimulates glutaminolysis.[42] Myc has a vital role in regulation of glutaminolysis. In addition to the above said genetic alterations in metabolism of tumour cell, abnormal tumour microenvironment has also play a key role. Tumour metabolism and the microenvironment have dynamic and complex relation and various studies have shown it and further research is going on for a better understanding. The regulation of this metabolic flexibility is poorly understood and will require a much greater degree of understanding, if effective therapeutic strategies targeting metabolism, are to be developed and effectively deployed.

Metabolites as a diagnostic marker

Metabolite markers are different from traditional biomarkers such as biochemical indices and/or protein-based markers, as they very much rely on analytical methods.[43] Metabolomics uses analytical instruments to profile metabolites present in clinical samples such as blood, urine, stool, and tumour tissue, which identifies key metabolite markers capable of discriminating among different groups. To date, a number of metabolite markers are reported from various clinical metabolomics studies, being developed or integrated as potential diagnostic modalities. Significant changes in carbohydrate metabolism in blood were revealed by metabolomic studies in patients with ovarian cancer and acute myeloid leukemia (AML).[44,45] One representative "oncometabolite" comprehensively studied is 2- hydroxyglutarate (2-HG), a product of IDH1/ IDH2 mutations, which was identified in many kinds of cancer including AML, breast cancer, renal cancer, intrahepatic cholangiocarcinoma, papillary thyroid carcinoma, etc.[46-53] High level 2-HG in blood was also reported in AML and breast cancer associated with poor prognosis, suggesting that it can also serve as an effective prognostic marker.[46,54] Fatty acids are another group of metabolites associated with carcinogenesis. Serum levels of unsaturated free fatty acids were revealed to be diagnostic indicators of early-stage colorectal cancer.[55] A nutrition intervention study reported higher intakes/blood levels of the Omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), relative to the Omega-6 arachidonic acid (AA) are associated with reduced breast cancer risk.[56]

Amino acids, a group of important molecules maintaining the physiological state of the biological systems, also play important roles in cancer development.[57] A recent metabolomics study showed that lowered aspartate in blood is a metabolic feature of human breast cancer.[57] Branched-chain amino acids including leucine, isoleucine, and valine, were found at high levels in blood in patients with human pancreatic adeno- carcinoma.

Bile acids, the main components of bile, were recently discovered to play important roles in tumor regulation.[58-59] Several hydrophobic bile acids may collaboratively promote liver carcinogenesis.[58] A recent metabolomics study identified a panel of five metabolites including glutamate,

choline, 1,5- anhydro-d-glucitol, betaine, and methylguanidine in plasma, which collectively can distinguish PC patients from healthy controls.

Metabolite as a diagnostic marker in cervical cancer

Identification of various metabolites and biomarkers play very important role in the understanding of beginning and progression of a cancer. Around 80% cervical cancer cases are reduced in the countries where enough resources to detect and treat precancerous lesions are available to women.[16]Worldwide, so far, the specific metabolic alterations involved in the progress of cervical cancer have not been completely identified. More efficient treatments are required for improved non-invasive early diagnosis of cervical cancer so that morbidity and mortality get reduced. Despite new diagnostic approaches, the definite diagnosis of this malignancy continues to be challenging. Fortunately, high-throughput metabolomics has been used to explore particular metabolites as potentially diagnostic and prognostic biomarkers for a deep understanding of diseases.

Squamous cervical cancer (SCC) can alter the level of certain small molecular metabolite in plasma through modulating gene expression. In a study done by Qun Liang *et al.*(2014)[60], they found that 3-methylhistidine, citric acid, cytosine, indoleacetic acid, salicyluric acid, L-methionine, aminomalonic acid, glutaric acid, ursodeoxycholic acid and N-acetylornithine, are involved in metabolic pathways such as the citrate cycle, lysine degradation, tryptophan metabolism, cysteine and methionine metabolism for the implication of these compounds in metabolic routes that may be associated with the early genesis of cervical cancer, which highlights their potential use as prognostic markers for the identification of women at risk of developing cervical cancer. [60]

Yin *et al.* identified two metabolites, phosphatidyl choline (PC) and lysophosphatidylcholine (LPC), via ultraperformance liquid chromatographic-mass spectrometry(UPLC-MS).Both metabolites were found considerably down- and upregulated in plasma of SCC as compared to uterine fibroid (UF) patients.[61]

Ye N *et al.* used 1H nuclear magnetic resonance (NMR)-based meta-bonomics to describe the metabolic profiles of cervical intraepithelial neoplasia (CIN) and cervical squa-mous cell carcinoma (CSCC). They found higher levels of VLDL, acetone, unsaturated lipid and carnitine, together with lower levels of creatine, lactate, isoleucine, leucine, valine, alanine, glutamine, histidine, glycine, acetylcysteine, myo inositol and choline in the plasma from patients with CIN. Patients with CSCC had higher levels of acetate and formate, together with lower levels of creatine, lactate, isoleucine, leucine, valine, alanine, glutamine, histidine and tyrosine. These results may be used in the identification of probable early diagnostic biomarkers of the cervical cancer.[62]

CONCLUSION

Around 90% of cervical cancer deaths occur in low and middle income countries, signifying the urgent need for cervical cancer prevention programmes. Prevention of cervical cancer very much relates to the prognostic and predictive biomarkers. To save many lives these markers are very important, as they help in taking clinical decisions at earliest. Metabolomics is becoming a sensitive profiling mean in search of potential biomarkers for cervical cancer detection. Metabolite profiling could become an essential ingredient of screening and triage for cervical cancer risk, par-ticularly in low and middle income countries, where low-cost HPV testing and cytological examinations are yet to be adopted at a large scale. Metabolic profiling technologies for metabolomic discoveries are expected to be standardized in future so that transformed cellular metabolism will be considered as a distinguishing element of cervical cancer. Metabolite profiling may allow us to extend diagnostic tests of cancer, and metabolite derivatives can be used for the molecular imaging of cancer. That will help in the development in novel remedial approaches for cervical cancer.

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