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GENETIC AND MOLECULAR BASIS OF TESTICULAR CANCER: A REVIEW

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ABSTRACT

Testicular cancer occurs in male reproductive part testicles it produces sperm and sex hormones in comparison of other type of cancer this is a rare one. The 90% of testicular cancer starts in germ cell (cells which makes sperms). So the germ cell tumor of testicles are classified into seminomas and non-seminomas, both of them occurrence rate are equal few testicular cancer consist both non-seminomas and seminomas. The pathogenesis and differentiation associated with molecular mechanism of testicular cancer can be understood through their associated genes, chromosomal abnormalities and molecular mechanism for the development of cisplatin acquired resistance. The epigenetics is also a important factor for testicular cancer it inherit the genetic factors which do not depends on genetic sequence variation but associated with expression and regulation of genes through methylation, modification of DNA protein histone. The studies associated with testicular cancer had shown that tumor cells DNA exhibit hypomethylation in comparison to normal cells. The molecular mechanism associated with cisplastin resistance and sensitivity is also explained.

KEYWORD: Testicular cancer, Sex hormones, Seminoma, Genetics, Epigenetics.

INTRODUCTION

Testicular cancer reports 1% for human malignancies in males and its incidence is increasing worldwide in last fifty years. This cancer develops in male reproductive part which results lump in testicles, swelling or pain in scrotum. Testicular cancer occurs mainly in germ cell accounts for more than 95% of testicular malignancies called as testicular germ cell tumors. Among various tumor associated with germ cells are predominantly common and are classed into two categories seminomas tumors and non- seminomas tumors (cancer that develop from sperm or eggs cells and tumors which are made up of more than one type of cell respectively) other type include sex-cord, stromal tumor and lymphomas. Testicular cancer is usually curable and treatable, radiation therapy, stem cell transplantation, chemotherapy are the treatment options. Even in severe cases treatment is possible cure rate is higher than 80% for this cancer. Diagnosis for this cancer is based on ultrasounds, physical examination and blood tests. It occurs in males of 20-34 years old very rare before 15 years old males.

The main etiology of testicular cancer is clearly not known but the there are few risk factors responsible for developing testicular cancer which are undescended testicles (testicle which has not moved properly into scrotum before birth), sexual disorders. It is assumed that presence of tumor results in undescended testicles and conjunction of both undescended testicles and tumor leads enlargement of tumor. Other genetic diseases are also the risk factor for this cancer.

Genetic factors associated with testicular cancer

The association of genetic factors with testicular cancer has been acknowledged widely. Through the population based study which was conducted in year 2000. It revealed that in a family if any one consist history of testicular cancer prone to the disease 8to10 fold than population without this factor of testicular cancer also a father affected with testicular cancer increases the chances of causing disease in his child from 6-10 folds.[1-3] Testicular cancer has been raised as factor mostly associated with neoplasm and the genetic factors next to thyroid and endocrine glands. The recent study evaluate that monozygotic and dizygotic twins individually which revealed esteemed intimate risk of heritability for testicular cancer.[4]

Beside all these evidence regarding the genetic background of testicular cancer development, the requirement of relevant study providing facts of genetics in individual containing testicular cancer associated family history is the main provocation. Crockford et al conducted the study of linkage in pedigree family of 237 among them one or few case history with testicular

cancer it shown 6 regions of interest on chromosomes as susceptibility loci by the technique chromosome conformation capture analysis.[5] It is associated with analyzing chromatin spatial arrangement in cells of testicular cancer function in chromatin interaction between proneness of target gene and SNPs which shown 3 feasible process of pathogenesis. Specifically risk associated 10 of the loci consisting genes involved with cell development related transcription regulation like GATA4 and 1 gene. They both function as a transcription factor responsible for postnatal testicular development, specification and differentiation. Risk associated with alleles polymorphisms of these genes were previously involved in progression of tumors.[6,7] PRDM14 and DMRT1 both gene were also found to be associated significantly and involved in sex determination and specification of germ cell.

The SALLA4 is a novel marker of germ cell in detecting testicular cancer it works through disruption of POU5F1 binding motif and further involve in conserving the pluripotency of embryonic stem cell.[8,9,10] Additionally five testicular cancer loci are found to be involved with genetic variation in pre-specified gene (candidate gene) with functionality in chromosomal assembly and microtubule assembly specifically TEX14 gene is responsible for assembly of kinetochore in the TGCTs.[11] Microtubule organization in interphase require essential proteins which is provided by WDR73 gene.[12] Few testicular cancer risk loci had delimit the KIT-MAPK cell signaling major role, Litchfield et al established the evidence regarding KIT gene is a main somatic driver of testicular cancer development.[13] All of these clearly reveals the pathogenesis of testicular cancer relies on genetic factors on wider scale. Recently copy number variations (CNVs) role in cancer development specifically testicular cancer have given spark to the genetic factor and testicular cancer association.[14,15]

The investigation in this respect was conducted for analyzing involvement of E2F-1 gene and CNVs as a risk factor for testicular cancer development. As E2F-1 protein is a regulatory transcription factor for transition of G1 to S-phase in cell cycle by interacting with tumor suppressor gene pRb (retinoblastoma). Deregulation of E2F-1 –pRb binding elevate the access of E2F-1 for binding its target gene, it increase susceptibility of tumor development.[16] In a study group of 261 patients consisting testicular germ cell history and control of 165 samples were studied which shown duplication of E2F-1 gene only in testicular germ cell patients. This was involved with elevated expression of E2F-1 in tumor specimen only procure from the patients consisting 3 E2F-1 copies. Although, non-tumor specimen shows lower level expression of E2F-1 and downstream mTOR phosphorylation.[17] These findings suggested involvement of E2F-1 and CNVs in susceptibility of testicular germ cell tumor (TGCT) with Akt/mTOR pathways of cell signaling. This tumor arise through transformation of germ cells, transformed germ cell exhibit pluripotency to differentiate in embryonic, extra embryonic and somatic tissue and also highly sensitive to cisplatin-based chemotherapy.

TGCTs contain various subtype as of microscopic anatomy in addition of seminomas and non-seminomas which are formed by extra gonadal and gonadal sites.[18] Both of them are two main subtype of TGCTs and contain distinct biological features and potential of metastasis, on other side non-seminomas function in differentiation of chorion, yolk sac, allantois the extra embryonic membrane and also embryonic differentiation its patterns encompasses, primordial zygote and phenotypes such as choriocarcinoma of extra-embryonal differentiation and tumor associated with yolk sac, operating a higher susceptibility for initial development and a imperfect diagnosis in advanced stage. The seminomas and non-seminomas tumors both shows capability of invasiveness they immerse by the common ancestor, which is carcinoma in situ where tumor cells arise and restricted in semniferous tubules.[19,20]

The non-seminomatous consist embryonal carcinoma, it has resemblance with stem cell and had the capacity to differentiate in various somatic lineages and the seminomas tumor has similarity with primordial germ cells and carcinoma in situ cells of forming TGCTs as a model for revealing gamete formation and development of germ cell in cancerous and normal system. In fact TGCTs associated with pathogenesis initiation is reported which occurs in uterus at the time of development of embryo with germ cell neoplasm non-differentiation or carcinoma in-situ development, it shows the primitive lesions. It is accomplished by dormancy interval which stops after puberty, postpubertal TGCTs emerged and suggests the testicular cancer can occur through hormonal burst. Wicha et al reported that testicular tumor originates and revealed the neoplastic cells keep stem cell characteristic arises from testicular tumors.[21]

Abnormalities and polymorphism of testicular cancer

Molecular abnormalities of testicular cancer includes gain of function or loss of function in few particular chromosomal region like the presence of abnormalities of chromosome arms 12p and their increase in number of copies. This is present in entire germ cell and involved in early stage of transformation in malignant tumors.[22] The molecular process of progression in germ cell is not understood strongly. The vulnerability of TGCTs is caused due to genetic effects, the powerful association for testicular cancer is been reveled through (SNPs) in the kit-ligand gene at the 12q22 position. It correspond the elevation of disease risk at 2.5 fold, the kit-ligand gene is associated in various features of progenitor germ cell (PGC) mainly survival and migration types of development.[23] c-kit gene is expressed very strongly in gonocytes fetus and pediatric stage, this gene in human encodes activity of tyrosine kinase receptor associated in spermatogenesis, melanocytes, haemopoiesis.[24] The

ligand or stem cell factor of c-KIT oncogene KITLG is situated on 12q21.3.2 position of chromosome, It is important for carrying out the dimerization of c-KIT and autophosphorylation for signaling of c-KIT-KITLG activation of its targets for survival and proliferation.[25] Variation in KITLG sequence is amenable for susceptibility of developing testicular cancer had recently been documented. The risk of SNP represents the most persistent alleles in Caucasian population generally and lacking in black Asian population this explains ethnic distribution of testicular cancer. The pathway of KIT is proposed to be activated constituently in TGCTs due to mutation in KIT oncogene or overexpression.[26]

Beside c-Kit two other genes are identified as risk variants sprouty RTK signaling Antagonast-(SPRY4) and BAK-1 -associated receptor kinase (BAK1) genes. The c-kit /KITLG associated genes participates in survival of germ cell and gonodal development in initial stages.[27,28] Genes SPRY4 is positioned at chromosome no.5 and it work as an inhibitor for protein kinase pathway involved with testicular tumor on the other side BAK1 involved with neoplasia function for promoting apoptotic factor it get halted through KITLG-KIT pathway. SNP related study in BAK-1, DMRT1 and KITLG shown the risk variants for both bilateral and familial testicular germ cell tumors.[29] The enzyme 17- β hydroxydehydrogenase-4 associated polymorphism is accountable for conversion of androgen to estrogen which is involved in TGCTs.[30,31] Additionally cytochrome P450 Cyp1A1 gene associated polymorphism encodes a hormone metabolize the protein, which have been recognized and correlated with vulnerability of TGCTs development.[32] Other most studied gene in respect of polymorphism is androgen receptor (AR) this gene is situated at Xq11q12 position and it shows two polymorphic regions situated at trans-activation domain in combination of codons for glutamine and glycine CAG and GCN respectively.[33] These trinucleotide repeats of polymorphic form changes leads to amend the AR associated transactivation and this results in seminoma development associated risk and carcinoma in-situ associated progression very strongly.[34] The suggestion had been made that all these sequences of polymorphic form presence may be associated with increased risk of TGCTs. However particular SNPs are recognized with development of TGCTs.[35] The transcription factor DMRT1 belongs to DNA binding gene family had a powerful implication in the testicular development among vertebrates and also involved in development of tumor with variation in genetics have strong relationship with susceptibility of developing TGCTs, expressed as pluripotent gene in testicular cancer. Other pluripotent gene NANOG and POU5F1 plays role in gametogenesis, differentiation and in tumor pluripotency.[36]

Epigenetic of testicular cancer

Beside genetic factors epigenetic mechanism is also an important factor involved in testicular cancer development. Epigenetics is measure of changes associated with alteration in gene expression without any changes in the sequence of DNA. It depends on histones post-transductional modification or DNA methylation. The inheritance needs transmission through germline in epigenetic patterns among generations. Programming of epigenetic occurs at the time of embryonic development in sex-selective manner sex from germline. In males after puberty spermatogenesis when become fully functional leads to epigenetic modification along with various steps of spermatogenesis differentiation from spermatogonia to spermatozoa in testicular germ cell. Infact, the demonstration of various spermatogenesis steps shows effective epigenetics associated modifications. These changes revealed that expression of various enzyme are involved in modification. The enzyme DNA methyltransferases (Dnmts) and histone methyltransferases (HMTs) are expressed in spermatogonia, and spermatocyte levels respectively. Hyperacetylation of H4 histone plays important role in histone removal and during spermatogenesis they are replaced by protamines. The environmental factors have the capability to alter the programming of epigenetic, it leads to the impact on offspring development. The alteration in epigenetic mechanism is one of the factors of TGCTs, gene methyaltion alteration in relation of tumor suppressor genes is common and important for tumorigenesis. Abnormality of hypermethylated CpG islands are found in every tumor including TGCTs. DNA hypomethylation of oncogenes leads DNA overexpression and finally to carcinogenesis.[37,38]

The cases of embryonal carcinogenesis revealed the methylation of intermediate type and seminomas, gonadoblastoma have higher DNA methylation. Abnormalities in the regulatory gene promoters region, results in silencing of gene expression all of this leads progression towards testicular cancer. The tumor suppressor genes hypermethylation of promoter region at CpG islands is an important process of gene inactivation.[39] Genes of adult are methylated at promoter region in cancer and are found to be unmethylated in embryonal carcinomas cells. The epigenetic associated studies in TGCTs cases revealed that DNA methylation is important for germ cells development and this modification is associated with DNMT3a and 3L associated with de novo methylation throughout the development of prenatal stage in germ cell. In males after birth DNMT1 and DNMT3b are involved for methylation and proliferation maintenance in spermatogonia.[40] DNMT1 in embryonal carcinoma is found to be upregulated and DNMT3a is highly expressed in testicular tumor in contrast to non-tumor testicular tissues.[41,42] DNMT3b is considered as predictive marker elevated risk of patients became critically worse with seminomas of stage-1. The tumors of non-seminoma type consist overexpressed DNMT3L,

indicates a new and important factor for human embryonic cells growth its supression results in inhibition of growth with resulting increase in methylation in LINE1 sequences.[43]

The intensity of methylation difference in seminomatous and nonseminomatous in testicular tumors is obtained from methylation of repetitive elements in DNA mainly Alu transposable elements.[44] Demethylation in transposable elements intensity is greater in seminomas than in non-seminomas. The degree of demethylation in LINE1 and Alu was evidently higher in testicular germ cell tumor than in tissue other than reproductive tissue (somatic tissue) of cancer cells. The special methylation at repetitive elements of DNA patterns are present in seminomas it speculate TGCTs genesis and their nature of pluripotentency inspite of DNA demethylation seen globally in cancer. The seminomas tumors and non-seminomas tumor contains LINE1 hypomethylation in DNA is because of epigenetic associated PIWI-interacting RNAs (piRNAs) inhibition which is group of non-coding small RNAs mainly present in lineage of germ cell and translated through the genome consisting transcribed repetitive, and transposable elements and various proteins of Argonaute (PIWIL1, PIWIL2, PIWIL4).[45] Undifferentiated germ cell tumors, early fetal germ cell and contains similar pluripotency of Nanog and Oct3/4 expression act as transcription factors. The regulation associated with the protein Oct4 and p53 are reciprocally homeobox containing pluripotency network and transcription factor and is predominantly responsible for pluripotency maintenance and resumption of undifferentiated stem cells of embryo and differentiation mediated suppression.[46] This can be detected in seminomas, embryonal carcinoma of germ cell and carcinoma cells, Nanog is not seen in somatic or adult cells of testis and their promoters leads to hypomethylation in spermatogonia and hypermethylation in sperm.[47] Methylation of CpG sites promoter can be silenced by OCT3/4-SOX2 associated expression of Nanog. DNA methylation in humans at different promoter elements is capable for inducing the Nanog expression silencing. Disregulation of DNA methylation gives an alternate mechanism of genetics for suspectibilty of TGCTs of familial types. In white blood cells (WBC) upregulated level of methylation at promoter site is seen in PDE11A, SPRY4 and BAK1 genes and down regulation of KITLG gene these all are associated in TGCTs of familial type.[48]

All of these changes associated with methylation of promoter may inhibits PDE11A, SPRY4, BAK1 and activate KITLG pathway further non seminoma tumors involve hypermethylation in several genes (MGMT, HIC1, APC, FHIT). Tumors which are sensitive shows hypermethylation in MGMT involve removal of DNA adduct and RARB involved in Retionic acid signaling. Other tumors which are resistant had hypermethylated HIC1 promoters and RASSF1A[49] Modifications of epigentics also occurs during spermatogenesis through various histone methyltransferase family which mediates histone3 lysine9 associated dimethylation or trimethylation. The family members of p63, p73, p53 genes plays role in tumors of germ cells. The unique expression of p63 isoform is observed in testis of humans and apes, epigenetic regulation leads 70-100% of all invasive tumor which assumed that these proteins works as proto-oncogenes in germ cell.[50] Non-seminoma consist few gene involved in activating histone H3-H4 methylation and few gene contain silencing of H3K9 this is observed in various subtypes revealing that would leads in abnormal expression of gene in the testicular tumor subtype. Additionally carcinoma in situ revealed the existence of low level of Histone H3K9me2 and H3K27me3 leads to repressive modification with higher levels of Histone acetylation and methylation at H3K9 and H3K4 respectively.[51]

Testicular cancer sensitivity and resistance associated molecular mechanism

Testicular cancer cure rate is more than 80% due to its efficacy towards cisplatin treatment. The chemoresistance development in these tumors not only revealed several authors to concrete on the molecular mechanism associated with resistance but also to develop constructive treatment for the pattern of invasive cancer of somatic tissue. Chemosenstivity is a native factor of tumors it is not associated with particular drug or its utilization it depends on the capability of sensing the destruction for activating the DNA Damage response (DDR). Which further function through encountering apoptosis, inhibition of DNA repair process and regulation of cell cycle in testicular tumors and rearrangement of DNA happens specifically in machinery of DNA repair. Where due to lower level of DNA repair protein expression renders its sensitivity for tumor cell towards drug.[52,53] Non-seminomas show higher resistance towards chemotherapy and higher excision repair cross complementation group 1 (ERCC1) protein in comparison of tissues of seminoma.[54] The higher level of ERCC1 is seen in cisplatin resistance cell lines and early stages of testicular cancer samples in comparison with cisplatin sensitive counterparts.[55] The DNA repair element downregulation may participate in chemosenstivity the way through which cell sensed the DNA damage and p53 response to it both are responsiveness of chemotherapy.

p53 mutation is seen in almost all type of cancers, inactivated by non-genomic mechanism functionally in the remaining cancers. It is not found to be mutated in testicular cancers and activates various chemotherapeutic agents exposure and the events for performing chemosensitivity of various tumors.[56] As it is a proto-oncogene works in apoptosis and regulation of cell cycle the two mutually exclusive cellular events.[57] In testicular cancer p53 retain wild type after non-genomic neutralization so it helps in resistance of drugs at the time of cell cycle arrest event or therapy associated with sensitivity. Infact the inability of these tumors of

repairing leads the effects of activating p53 activity downstream which enhance the burden of damage and further it activates apoptosis above cell cycle inhibition.[58]

Various facts have revealed that in exceptional TGCTs sensitivity apoptosis have a vital position towards cisplatin associated therapy because of its specific sensitivity towards p53 activation. The wild type p53 suppression in testicular germ cell tumors can eliminate the cisplatin sensitivity of take decision of apoptosis or cell cycle arrest resulting chemosenstivity and resistance. The TGCT sensitive cells leads to activation of p21 and HDM2 associated expression following cisplatin drug therapy.[59] So, p53 will able to interrelate with Oct-4 resulting an interplay between both of them which will be amenable for chemotherapy and retaliation of these tumors.

The embryonic transcription factor Oct-4 binds over DNA sequence with its POU domain it control the pluripotency and survival in embryonic stem cell where it is expressed in cooperation with distinct transcription factors like SOX-2. In TGCTs cells at lysine k123 position Oct4 sumoylation promotes hypoxia and downregulation of Oct-4 event is amenable for resistance associated in both cisplatin and bleomycin resistance in comparison peptidase SENP1 can sumolyate Oct-4 and improvise the TGCTs cells chemosenstivity. Rather than Oct-4 presence cellular context is responsible and liable for promotion of chemosensitivity in various tumors. Probably the absence of p53 in TGCTs and mutant dispossess the particular cancerous cells by properties promoting cancer like the capability of inducing high affinity transcription from promoter p21. The mutation in p53 gene are pronounced to increase reprogramming from normal to stem cell in Oct4 and Sox2 existence.[60] If p53 mutations is not present TGCTs can be constantly their but Oct-4 re-expression for supporting cancer formation by the pro-survival network of embryonic cell is the normal feature for testicular tumors. So, neither p53 nor Oct-4 leads chemoresistance rather both p53-oct-4 reciprocally works. These two factors balance with each other oct-4 is a core factor of stemness and recently established reprogramming of somatic cells associated integral component it stimulates stem cells pluripotency and p53 is responsible for blocking the stimulation of pluripotency. It results in negative effect of both on differentiation promotion, cell cycle regulation, stemness organization and stimulation of survival, it is accountable for apoptotic stimuli sensitivity retainment. Contradictorily, p53 and oct-4 associated mutations leads to resistance from cisplatin[61] TGCTs associated chemosentivity is done by cellular factors of TGCTs instead of Oct4. In TGCTs p53 mutation absence may be consistent imposed by Oct4 by re-expression for carcinogenesis through establishment of pro-survival network of embryonic cell which is representative feature for this tumor. TGCTs associated cisplatin treatment leads to downregulation of p21 G/M phase inhibition and then cell death through the apoptosis mechanism, which leads to miR32a upregulation.[62] Infact miR372 and 73 are induced with transcription factors Oct-4, Sox-2 and Nanog are overexpressed in TGCTs by inhibition of large tumor suppressor homolog (LATS2) expression a wild type p53 stimulation associated target gene. The p53 determines the cisplatin response but not alone with its other family member p63 or p73 can also play prominent role testicular cancer, mainly in the cases of p53 functionality is lost function by Noxa and Puma regulation or other regulator of p53 MDM2 may intervene. In testicular germ cell tumor the DNA mismatch repair works alongwith chemosenstivity connected to lower nucleotide excision repair activity. The resistance of tumor were involved with errors in mismatch mispair repair (MMR) and consist instability of microsatellite developed from low expression of MMR protein MLH 1,6,2[63] If there is loss of function in MMR then lesion such as BRAF mutation activation are the requirement of cells for its viability and which will lead to chemosenstivity. The capability of repairing DNA damage reduction is due to defects in homologous recombination and crosslink repair between two strands. The elevated level of p53 intratumoral level are mainly responsible for apoptotic factors Noxa, Puma in TGCTs higher level of BCl-2 exist. Therefore in both chemo sensitive and resistant cells of testicles, the pathways of Oct4/miR-106b/p21or p21 of cytoplasm are related directly in targeting the modulation and inhibition of tumor suppressor genes p53 and MDM2 interaction results to p53 pathway hyperactivation and stimulation of apoptosis influentially. Juliachs et al demonstrated that PDGRFβ–AKT pathway has major contribution for cisplatin resistance in testicular tumors.[64] The pathway phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase (PTEN) which can only the inhibitory enzyme of the PDGRFβ–AKT pathway is lost in almost all type of TGCTs.[65] On the other hand overexpression of AKT pathway has been analyzed in testicles of tumor cells which are sensitive to cisplatin in comparison of complementary cells containing sensitivity this is consequence of platelet-derived growth factor receptor beta (PDGFR-beta) and their associated ligand level increased along with protein levels and mRNA level .Likewsie, The effective combination of genomics, proteomics, transcriptomics, metabonomics explore the defective mechanism of genes, proteins, RNA and metabolic product by which prognosis and treatment of cervical cancer become possible.[66] Cell signaling also place a major part in all of these process, without it not a single cell would respond. Hence, will lead to uncontrolled cell division and cell cycle leading to finally causation of cervical cancer and other types of cancers.

CONCLUSION

Testicular cancer is prominent in males consisting higher rate of prevalence it occur mainly at the reproductive age. This cancer mainly forms in reproductive part of males and most of the cancer of this type arise in germ

cell so named as testicular germ cell tumor. The progression associated with this caner had been increased in last few decades. The molecular mechanism associated with disease can be explained mainly with its genetics, epigenetics and a chromosomal abnormality all of these factors leads the complete understanding of this cancer mechanism. The etiology of Testicular cancer is not very much clear but the risk factor involved with it is cryptorchidism, sexual disorders and several other. The novel strategy of investigation is important for clarifying progression of testicular germ cells tumors also for further prognosis and diagnosis.

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