RINGKASAN

Analisis Molekuler Patogenesis Karsinoma Sel Skuamosa Rongga Mulut Berdasarkan Pola Mutasi Gen p53 Dan p16

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Karsinoma sel skuamosa rongga mulut (KSSRM) adalah jenis kanker rongga mulut yang berasal dari jaringan epitel keratinosit rongga mulut yang terjadi akibat mutasi gen spesifik pada gen regulator. Gen supresor tumor (GST) p53 dan p16 merupakan target gen regulator spesifik yang penting pada kontrol siklus sel kanker dan yang paling sering mengalami mutasi pada berbagai kanker termasuk KSSRM melalui jalur yang berbeda. Frekuensi mutasi GST p53 dan p16 bervariasi di berbagai negara dan populasi etnis di dunia. Berbagai penelitian sering menghubungkan adanya mutasi GST p53 dan p16 dengan parameter klinikohistopatologis seperti umur, jenis kelamin, lokasi tumor, faktor resiko perokok/penginang, status TNM, stadium klinis, KSSRM diferensiasi sel baik dan jelek.

Tujuan penelitian ini adalah mengungkap patogenesis molekuler karsinoma sel skuamosa rongga mulut melalui pola mutasi gen supresor tumor p53 dan p16 serta hubungannya dengan gambaran klinis dan histopatologis. Pada penelitian ini dilakukan observasional analitik laboratorik dengan pendekatan biologi molekuler terhadap data yang bersifat *crosssectional*. Sampel penelitian: jaringan KSSRM; n=40, usia 40 th sampai > 60 th dan kontrol; jaringan normal, n=16, usia 18-25 th, diambil dari populasi penderita normal dan KSSRM yang datang ke Laboratorium Bedah Mulut dan Poliklinik Onkologi THT RSUD. Dr. Sutomo. Pada jaringan KSSRM dan kontrol dilakukan optimasi PCR dan analisis PCR-SSCP dengan pengecatan perak nitrat. Hasil deteksi mutasi GST p53 didapatkan dari mutasi GST p53 ekson 5 dan atau ekson 7 sedangkan mutasi GST p16 didapatkan dari mutasi GST p16 ekson 1 dan atau ekson 2.

Data penelitian dilakukan analisis untuk membedakan kejadian mutasi pada GST p53 dan p16 pada KSSRM dengan menggunakan *Cochran's Q Test*. Hasil uji beda antara mutasi GST p53 sebanyak 28 kasus 28/40 (70%); ekson 5, 11 kasus 11/40 (27,5%); ekson 7, 22 kasus, 22/40 (55%) disertai mutasi heterozigot 22 kasus 22/22 (100%) dengan GST p16 sebanyak 14 kasus 14/40(35%); ekson 1, 14 kasus 14/40 (35%) disertai mutasi homozigot 10 kasus 10/14 (71,4%); ekson 2, 6 kasus 6/14 (15%) terdapat perbedaan signifikan (p=0,002).

Data penelitian untuk analisis uji hubungan kejadian mutasi pada GST p53 dan p16 pada KSSRM dengan parameter klinikohistopatologis menggunakan *Contigency coefficient*. Hasil uji hubungan antara umur 40-50 th (36,4%), 51-60 th (75%), >60 th (90%) dengan mutasi GST p53 (70%) didapatkan hubungan signifikan (p=0,007) terutama p53 ekson 7 (55%); p=0,006 sedangkan hasil uji hubungan antara umur 40-50 th (36,4%), 51-60 th (35%) >60 th (33,3%) dengan mutasi GST p16 (35%) tidak didapatkan hubungan signifikan (p=0,990). Hasil uji hubungan antara perokok/penginang (82,1%) dan tidak perokok/penginang (41,7%) dengan mutasi GST p53 (70%) pada KSSRM didapatkan hubungan signifikan (p=0,010) terutama p53 ekson 7 (55%); p=0,013 sedangkan hasil uji hubungan antara mutasi perokok/penginang (42,9%) dan tidak perokok/penginang (16,7%) dengan mutasi

GST p16 (35%) pada KSSRM tidak didapatkan hubungan signifikan (p=0,112). Hasil uji hubungan stadium klinis I-II (42,9%) dan III-IV (84,6%) dengan mutasi GST p53 (70%) pada KSSRM didapatkan hubungan signifikan (p=0,006) terutama p53 ekson 7 (55%); p=0,014 sedangkan hasil uji hubungan stadium klinis I-II (28,6%) dan III-IV (38,5%) dengan mutasi GST p16 (35%) pada KSSRM tidak didapatkan hubungan signifikan (p=0,532). Hasil uji hubungan pada penderita KSSRM diferensiasi sel baik (60%) dan jelek (80%) dengan GST mutasi p53 (70%) tidak didapatkan hubungan signifikan (p=0,168) kecuali mutasi p53 ekson 5 (27,5%) terdapat hubungan yang signifikan dengan penderita KSSRM diferensiasi sel baik dan jelek (p=0,013) sedangkan hasil uji hubungan KSSRM diferensiasi sel baik (20%) dan jelek (50%) dengan mutasi p16 (35%) terutama p16 ekson 1 (35%), p=0,047.

Kesimpulan hasil uji beda dan hubungan antara mutasi GST p53 dan p16 pada KSSRM menunjukkan GST p53 paling sering terlibat dalam mekanisme patogenesis molekuler KSSRM dan tidak terpengaruh aktivitasnya oleh peran GST p16 dalam jalur siklus sel KSSRM, terdapat hubungan yang signifikan dengan umur (p=0,006) menunjukkan mutasi p53 lebih sering terjadi pada usia tua terutama pada ekson 7, pengaruh perokok/penginang (p=0,013) merupakan faktor resiko pemicu perubahan genetik spesifik pada KSSRM, GST p53 paling banyak ditemukan pada stadium lanjut, terutama pada ekson 7 dan berhubungan dengan transformasi fenotip keganasan (p=0,014) dan tidak terdapat hubungan yang signifikan (p=0,525) dengan KSSRM diferensiasi baik maupun jelek kecuali p53 ekson 5 (p=0,013) sehingga GST p53 ekson 7 dapat dijadikan target diagnosis dan prognosis molekuler KSSRM dan merupakan daerah *hot spot* mutasi p53 yang mempunyai hubungan signifikan dengan gambaran klinis KSSRM dan p53 ekson 5 dapat dijadikan target diagnosis dan prognosis molekuler KSSRM dan merupakan daerah *hot spot* mutasi p53 yang mempunyai hubungan signifikan dengan gambaran HPA KSSRM. Gen supresor tumor p16 tidak didapatkan hubungan yang signifikan dengan umur (p=0.990), perokok/penginang (p=0,112), stadium klinis (p=0,532) kecuali terdapat hubungan signifikan dengan KSSRM diferensiasi sel baik dan jelek terutama pada ekson 1 (35%), p=0047. Hal ini menunjukkan GST p16 ekson 1 memegang peranan penting dalam progresivitas KSSRM dan dapat dijadikan target indikator diagnosis dan prognosis molekuler spesifik pada KSSRM.

SUMMARY

The Molecular Pathogenesis Analysis of Oral Squamous Cell Carcinoma Based on The Pattern of Tumor Supressor Gene p53 and p16 Mutations

Peter Agus

Oral squamous cell carcinoma (OSCC) is a type of oral cancer which derives from epithelial tissue oral keratinocytes which had mutation gene in specific key target regulatory genes. The tumor supressor gene (TSG) p53 and p16 are a specific key target regulatory genes in cell cycle control which had most frequently mutations in several human tumor including in OSCC via different pathway. The incidence of TSG p53 and p16 mutations in OSCC were found varied in several countries and ethnic population in the world. Several studies have also been associated the presence of TSG p53 and p16 mutations with clinicopathological parameters such as age, gender, tumor location, risk factor of betel quid chewing or cigarette smoking habits, TNM status, clinical stage, well differentiated and poorly differentiated OSCC.

The purpose of this studies is to elucidate molecular pathogenesis of OSCC through the pattern of TSG p53 and p16 gene mutations also associate with clinic and histopathologic feature. Analitycal observations laboratoric comparative study using cross sectional design was used with molecular biology approach. Research sample: OSCC tissue; n=40; age 40 yr till over 65 yr and control patients; normal tissue, n=16; age 18–25 yr which were taken from population of normal and OSCC patients visiting to the laboratory of oral surgery and out patients clinic in Oncology ENT RSUD Dr. Sutomo. In this study, PCR optimation and PCR-SSCP analysis with silver nitrat staining were done for detection of TSG p53 and p16 mutations in OSCC and normal tissue. The result of TSG p53 mutations was obtained from exon 5 and or exon 7 of p53 gene mutations, while TSG p16 mutations was obtained from exon 1 and or exon 2 of p16 gene mutations.

Research data were analyzed for differencies between TSG p53 and p16 mutations using Cochran's Q test showed p53 gene mutations were detected in 28 cases 28/40 (70%): 11 cases 11/40 (27,5%) in exon 5; 22 cases 22/40 (55%) with heterozygous mutation 22 cases 22/22 (100%) in exon 7 and p16 gene mutations were found 14 cases 14/40 (35%): 14 cases 14/40 (35%) with homozygous mutations 10 cases 10/14 (71,4%) in exon 1; 6 cases 6/40 (15%) in exon 2, a statistically significant difference were found between the incidens of TSG p53 and p16 mutations (p=0,002).

Research data were analyzed for associates between clinicopathological parameters and the incidens of TSG p53 and p16 mutations using Contigency coefficient showed the incidens of TSG p53 mutation (70%) was significantly associated with age 40-50 yr (36,4%), 51-60 yr (75%), >60 yr (90%); (p=0,007) especially in exon 7 of p53 gene (55%); p=0,006, while the incidens of TSG p16 mutation (35%) was not significantly associated with age 40-50 yr (36,4%), 51-60 yr (35%), >60 yr (33,3%); (p=0,990). The insidens of p53 gene mutation (70%) was significantly associated with betel quid chewing or cigarette smoking (82,1%) and no betel quid chewing or cigarette smoking (41,7%); (p=0.010) especially in exon 7 of p53 gene (55%); p=0,013 while the incidens of p16 gene mutation (35%) was

not significantly associated with betel quid chewing or cigarette smoking (42,9%) and no betel quid chewing or cigarette smoking patients (16,7%); (p=0,112). The insidens of p53 gene mutations (70%) was significantly associated with clinical stage I-II (42,9%) and III-IV (84,6%); (p=0,006) particularly in exon 7 of p53 gene (55%); p=0,014 while the insidens of p16 gene mutations (35%) was not significantly associated with clinical stage I-II (28,6%) and III-IV (38,5%); (p=0,532). The insidens of p53 gene mutations (70%) was not significantly associated with well differentiated (60%) and poorly differentiated OSCC patients (80%); (p=0,168) with the exception in exon 5 of p53 gene (27,5%) was significantly associated with well differentiated and poorly differentiated OSCC patients (p=0,013) while the insidens of p16 gene mutations (35%) was significantly associated with well differentiated (20%) and poorly differentiated OSCC patients (50%); (p=0,047) especially in exon 1 of p16 gene (35%); p=0,047.

The conclusion of this study showed that the p53 gene are more frequently involved in molecular pathogenesis of oral cancer as compare to that of p16 gene especially in the cell cycle control of OSCC and its activity are not influence with the inactivity alteration of p16 gene, which was significantly associated with age (p=0,006) reveal that mutation of p53 gene occurs at older age, especially in exon 7 and the influence of betel quid chewing or cigarette smoking (p=0,013) can be as predisposition risk factor for the genetic alteration in OSCC patients; The p53 gene mutations especially in exon 7 of p53 was found frequently in advance stage and relationship with malignant phenotype transformation (p=0,014) which was not significantly associated with well differentiated and poorly differentiated OSCC (p=0,525) with the exception of p53 gene mutations in exon 5 (p=0,013). Thus, exon 7 of p53 gene could be as a molecular diagnostic and prognostic specific target indicator in OSCC and a hot spot region of p53 gene which was significantly associated with clinical status of OSCC, while exon 5 of p53 gene could be as a molecular diagnostic and prognostic specific target indicator in OSCC and a hot spot region of p53 gene which was significantly associated with histopathologycal status of OSCC. The p16 mutations was not significantly associated with age (p=0.990), betel quid chewing or cigarette smoking (p=0,112), clinical stage (p=0,532) with the exception of well differentiated and poorly differentiated OSCC especially in exon 1, 35% (p=0,047) which indicates that exon 1 of the p16 gene may play as role in the progresivity of OSCC and could be as a molecular diagnostic and prognostic specific target indicator in OSCC.

ABSTRACT

The Molecular Pathogenesis Analysis of Oral Squamous Cell Carcinoma Based on the Pattern of Tumor Supressor Gene p53 and p16 Mutations

Peter Agus

The purpose of this study is to determine the presence of the tumor supressor genes (TSG) p53 and p16 mutations, and to associate these mutations with the clinical and histopathological status of the OSCC patients in order to elucidate the molecular pathogenesis mechanisms of OSCC based on the pattern of p53 and p16 mutations. Analitycal observasional comparative study using cross sectional design was used. 40 untreated primary OSCC biopsy sample with varied histories of betel quid chewing or cigarette smoking and normal tissue biopsy material taken from 16 normal patients were analysed for the presence of mutation in the conserved region of the p53 gene (exon 5 and or 7) and the p16 gene (exon 1 and or 2) by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP).

The results of this study showed p53 gene mutations were detected in 28/40 (70 %): exon 5; 11/40 (27,5%) with heterozygous mutation 9/11 (81,8%), exon 7; 22/40 (55%) with heterozygous mutation 22/22 (100%) and p16 gene mutations were found 14/40 (35%): exon 1; 14/40 (35%) with homozygous mutation 10/14 (71,4%) and exon 2; 6 (15%) with homozygous mutation 4/6 (71,4%). A statistically significant difference was found between the incidens of TSG p53 and p16 mutations (p=0,002; Cochran's Q Test). The incidens of p53 mutation was significantly associated with age (p=0,007), status of cigarette smoking or betel quid chewing (p=0,010), clinical stage (p=0,006) which was not significantly with well and poorly differentiated with the exception in exon 5 of p53 gene (p=0,013) and the incidens of p16 mutation was not significant associated with age status of betel quid chewing or cigarette smoking, clinical stage, however exhibited significant associated with well and poorly differentiated of OSCC especially in exon 1 of p16 gene (p=0,047). All data were analized for associates between clinicopathological parameters and the incidens of p53 and p16 mutations using Contigency Coefficient.

This study concludes that :1) p53 gene are more frequently involved in molecular pathogenesis of OSCC as compare to that of p16 gene; 2) mutation of p53 gene especially in exon 7 occurs more frequently at older age of OSCC patients, 3) mutation of p16 gene especially in exon 1 occurs equally at older and younger age of OSCC patients, 4) betel quid chewing or cigarette smoking are predisposition risk factor for the acquirement of p53 gene mutation in OSCC patients, 5) betel quid chewing or cigarette smoking are not predisposition risk factor for the acquirement of p16 gene mutation in OSCC patients, 6) mutation of p53 gene especially in exon 7 may play an important role in malignant phenotype transformation of OSCC, 7) mutation of p16 gene may not play indirect in malignant phenotype transformation of OSCC, 8) mutation of p53 gene may not play in the progressivity of OSCC with the exception of mutation in exon 5 of p53 which indicates that in exon 5 of p53 gene may play as role in the progressivity of OSCC, 9) mutation of p16 gene especially in exon 1 of p16 gene may play as role in the progressivity of OSCC.

Key word *: TSG, p53, p16, mutation, OSCC, age, betel quid chewing, cigarette smoking, clinical stage, well differentiated, poorly differentiated.*