RINGKASAN

Ekspresi Protein c-erbB2, p53, pRb dan MIB-1 Pada

Karsinoma Duktal Payudara Insitu, Invasif dan Metastasis

Imam Susilo

Karsinoma payudara adalah tumor ganas yang banyak ditemukan pada perempuan. Tumor ini memiliki perilaku biologis yang heterogen, sehingga pengetahuan mengenai petanda tumor menjadi sangat penting untuk menentukan prognosis dan terapi terhadap penyakit tersebut. Penentuan diagnosis, prognosis dan pilihan terapi sampai saat ini, terutama masih didasarkan pada keadaan klinis dan morfologis, walaupun berbagai penelitian terakhir menunjukkan bahwa terdapat hubungan yang erat antara pertumbuhan karsinoma dengan kelainan yang terjadi pada tingkat molekuler yang sifatnya tidak tunggal. Target kelainan pada tingkat ini adalah gen regulasi sel yang terdiri dari gen pemicu pertumbuhan (*proto-oncogene*), gen penghambat pertumbuhan (*tumor supresor gene*), gen pengendali apoptosis (*progammed cell death gene*) dan gen yang termasuk dalam perbaikan DNA (*DNA repair genes*). Oleh karena itu diperlukan usaha untuk dapat menjelaskan perubahan yang terjadi pada tingkat molekuler sebagai landasan yang kuat untuk menentukan prognosis dan terapi karsinoma duktal payudara. Masing-masing gen tersebut menyandi protein tertentu (*onco-protein*) yang dapat mengendalikan aktivitas biologis sel kanker dan dapat dideteksi melalui metode pemeriksaan tertentu.

Tujuan penelitian ini adalah menjelaskan mekanisme perbedaan ekspresi protein c-erbB2 (*oncogene*), p53 (*apoptosis – repair gene*), pRb (*tumor supresor gene*) dan MIB-1 (*proliferation gene*) pada berbagai ukuran diameter tumor, diferensiasi, progresivitas pertumbuhan dan *grading* histopatologis karsinoma duktal payudara.

Rancangan penelitian ini adalah menggunakan *cross sectional study,* dengan jenis penelitian analisis observasional, dan pengambilan sampel secara random.

Tiga puluh sampel berupa jaringan tumor pada penderita karsinoma duktal payudara (NOS / NST) di Bagian / SMF / Instalasi Patologi Anatomi yang diterima dari Bagian / SMF / Instalasi Bedah Fakultas Kedokteran Universitas Airlangga / RSU. Dr. Soetomo Surabaya, dipilih dari keseluruhan sampel karsinoma payudara mulai dari Februari 2003 sampai Maret 2005, yang terdiri dari tiga kelompok, yaitu karsinoma duktal insitu 10 sampel, karsinoma duktal invasive 10 sampel dan karsinoma duktal metastasis 10 sampel. Penentuan besar sampel untuk keseluruhan kelompok didasarkan pada penelitian pendahuluan yang dilakukan sebelumnya. Kriteria inklusi penelitian ini adalah sampel pasien karsinoma duktal payudara di Bagian / SMF / Instalasi Patologi Anatomi yang diterima dari Bagian / SMF / Instalasi Bedah Fakultas Kedokteran Universitas Airlangga / RSU. Dr. Soetomo Surabaya, umur 30 – 70 tahun, perempuan, tidak menderita keganasan lain, tidak menderita infeksi secara klinis. Semua sampel pasien karsinoma duktal payudara dilakukan pemeriksaan ulang terhadap data dan morfologi untuk menentukan identitas, morfologi (makroskopis dan mikroskopis), diferensiasi, progresivitas pertumbuhan dan *grading* histopatologis karsinoma duktal payudara. Kemudian seluruh sampel dilakukan pemeriksaan biologik terhadap ekspresi protein c-erbB2, p53, pRb dan MIB-1.

Pemeriksaan imunopatologik dilakukan dengan cara menggunakan metode imunohistokimia, menggunakan antibodi monoklonal terhadap protein c-erbB2, p53, pRb dan MIB-1. Penilaian dan pengukuran variabel dari pemeriksaan imunopatologik dilakukan dengan cara menghitung jumlah sel per 200 sel tumor yang imunoreaktif pada permukaan sel untuk ekspresi protein c-erbB2, dan pada inti sel untuk ekspresi protein p53, pRb dan MIB-1. Pemeriksaan imunohistokimia untuk semua sampel dilakukan di Divisi Imunohistokimia Bagian Patologi Anatomi Fakultas Kedokteran Universitas Airlangga Surabaya.

Analisis yang digunakan dalam penelitian adalah analisis multivariat, dengan menggunakan program SPSS, yang dimaksudkan untuk mendapatkan perbedaan ekspresi protein c-erbB2, p53, pRb dan MIB-1 pada berbagai ukuran diameter, diferensiasi, progresivitas pertumbuhan dan *grading* histopatologis karsinoma duktal payudara.

Berdasarkan uji multivariat metode Wilks' Lambda menunjukkan bahwa ekspresi protein c-erbB2, p53, pRb, dan MIB-1 secara bersama berbeda terhadap berbagai ukuran diameter, diferensiasi, progresivitas pertumbuhan dan *grading* histopatologis karsinoma duktal payudara ($p=0,000 < \alpha=0,05$), kecuali ekspresi MIB-1 tidak signifikan terhadap berbagai *grading* histopatologis ($p=0,051 > \alpha=0,05$).

Uji multivariat dari variabel ekspresi protein c-erbB2, p53, pRb dan MIB-1 terhadap berbagai ukuran diameter karsinoma duktal payudara, menunjukkan bahwa protein c-erbB2 diekspresikan oleh tumor paling tinggi. Uji multivariat terhadap variabel ekspresi protein pada berbagai diferensiasi karsinoma duktal payudara, menunjukkan bahwa variabel protein c-erbB2 diekspresikan oleh tumor paling tinggi dan diikuti oleh protein p53, MIB-1 dan pRb. Sedangkan uji multivariat terhadap variabel ekspresi protein pada berbagai progresivitas pertumbuhan karsinoma duktal payudara, menunjukkan protein c-erbB2 diekspresikan oleh tumor paling tinggi dan diikuti oleh protein p53, MIB-1 dan pRb. Hal yang sama juga pada uji multivariat terhadap variabel ekspresi protein pada berbagai grading histopatologis karsinoma duktal payudara, menunjukkan bahwa variabel protein c-erbB2 diekspresikan oleh tumor paling tinggi dan diikuti oleh protein p53, dan pRb, sedangkan tidak demikian dengan protein MIB-1 yang kurang signifikan.

Analisis klasifikasi data pada berbagai pengelompokan penelitian (diameter, diferensiasi, progresivitas pertumbuhan dan grading histopatologis), menunjukkan bahwa sistem pengelompokan data berdasarkan diferensiasi sel tumor adalah yang terbaik. Hal ini dapat

ditunjukkan dengan hasil uji klasifikasi data pada pengelompokan penelitian, yang menunjukkan bahwa pengelompokan data berdasarkan diferensiasi terdapat sebanyak 22 kasus dengan diferensiasi buruk yang terklasifikasi dengan benar atau sebesar 73,3 %.

Dari penelitian ini dapat ditarik kesimpulan bahwa protein c-erbB2, p53, pRb, dan MIB-1 secara bersama diekspresikan berbeda secara signifikan terhadap berbagai ukuran diameter tumor, diferensiasi progresivitas pertumbuhan dan *grading* histopatologis (kecuali MIB-1 terhadap *grading* histopatologis dengan $p=0,000 < \alpha=0,05$). Perilaku biologis sel karsinoma duktal payudara yang tercermin dari tampilan klinis dan morfologis ditentukan oleh lesi genetik yang sifatnya tidak tunggal.

Diferensiasi sel tumor, ekspresi protein c-erbB2 dan p53 karsinoma duktal payudara adalah faktor prognosis terbaik yang mempunyai nilai signifikan paling tinggi pada penelitian dan diharapkan dapat digunakan untuk menentukan prognosis dan terapi yang sesuai terhadap pasien karsinoma duktal payudara.

SUMMARY

Protein Expression c-erbB2, p53, pRb and MIB-1 in Breast Cancer

Imam Susilo

Breast cancer is a malignant tumor mostly disclosed in women. It has heterogenous biological behaviour - so that the knowledge of tumor markers is very important to determine its prognosis and therapy. Up to now, the determination of prognosis and treatment of choice is still based on clinical and morphologic finding although recent studies pointed out that there was tight relationship between carcinoma growth and molecular abnormalities including normal cell gene consisting of proto-oncogene, tumor suppressor gene, programmed cell death and DNA repair gene. Therefore, the description of molecular changes is required - in determining the prognosis and therapy of breast cancer. Each of gene as oncoprotein can control the proliferation of cancer cells and be detected by a certain method of investigation.

The aim of study is to describe the different of protein expression c-erbB2 (oncogene), p53 (apoptosis – repair gene), pRb (tumor suppresor gene) and MIB-1 (proliferation gene) in various diameters, cell differentiation, progressiveness and grade of breast cancer.

The design of study used cross section study, analysis observational study, and random sample taking.

30 samples of tumor specimens from the patients with breast cancer, received from Surgical Department, at Pathologic Anatomic Department of Dr. Soetomo Hospital, Medical School, Airlangga University, Surabaya were randomly selected. The samples of breast cancer from February 2003 to March 2005, consisting of three groups, ie, 10 samples of in situ breast cancer, 10 samples of invasive ductal carcinoma, and 10 samples of metastatic ductal carcinoma. The determination of total number of all groups was based on the previous study. Inclusion criteria of study were samples from patient with breast

cancer received from Surgical Department at Pathologic Anatomic Department of Dr. Soetomo Hospital, Medical Faculty, Airlangga University, Surabaya; 30-70 years old ; female ; no other malignancies ; no clinical infection. The data and morphology of all samples of breast cancer patients were reexamined to determine identity, morphology (macroscopy and microscopy), the differentiation of tumor, tumor cell growth progression and grade. Then, to all samples of in situ, invasive, and metastatic ductal carcinoma were undertaken biologic investigation of protein expression c-erbB2, p53, pRb and MIB-1.

Immunopathologic investigation was carried out by using immunohistochemical method, with antibody monoclonal against protein c-erbB2, p53, pRb and MIB-1. The assesment and variable measurement of biologic investigation were conducted by quantifying a number of cells per 200 tumor cells which positively react in the surface of cells by protein expression c-erbB2,and nuclei by protein expression p53,pRb and MIB-1. Immunohistochemical examination of all samples was performed in the Immunohistochemical Division of Pathologic Anatomic Department, Dr. Soetomo Hospital, Medical School, Airlangga University, Surabaya.

Multivariate analysis using SPSS programe to obtain the difference of protein expression c-erbB2, p53, pRb and MIB-1 with various diameters, cell differentiation progressiveness and grade of ductal carcinoma was exerted.

Based on multivariate test of Wilks' Lambda method, protein expression of c-erbB2, p53, pRb and MIB-1 was concomitantly different in various tumor diameters of breast cancer ($p = 0,000 < \alpha = 0,05$). With methode of Wilks' Lambda method, protein expression c-erbB2, p53, pRb and MIB-1 was simultaneously different in various carcinoma cell differentiation of breast cancer ($p = 0,000 < \alpha = 0,05$) and with Wilks' Lambda method, protein expression c-erbB2, p53, pRb and MIB-1 was concomitantly different in various progressiveness of ductal carcinoma growth ($p= 0,000 < \alpha= 0,05$). Also with Wilks' Lambda method, protein

expression c-erbB2, p53, and pRb (without MIB-1) was concomitantly different in various grade of ductal carcinoma ($p = 0,000 < \alpha = 0,05$).

Variable multivariate test of protein expression c-erbB2, p53, pRb and MIB-1 in various diameters of ductal carcinoma revealed that protein c-erbB2 was expressed by most tumor; then followed by protein p53, MIB-1 and pRb consecutively. Multivariate variable test of various degree of differentiated of ductal carcinoma cell designated that variable of protein c-erbB2 was expressed by most tumor (the highest in moderate cell differentiation group); then, followed by protein p53, MIB-1 and pRb; whereas, variable multivariate test of various progressiveness of ductal carcinoma growth showed that there was similar result, ie, variable of protein c-erbB2 was expressed by most tumor (the highest in invasive group) and then, followed by protein p53, MIB-1 and pRb; also variable multivariate test of various grade of ductal carcinoma showed that similar result, ie, c-erbB2 was expressed by most tumor and then, followed by protein p53 and pRb.

It is concluded that protein c-erbB2, p53, pRb and MIB-1 simultaneously expressed are significanly different in various tumor diameters, cell differentiation, progressiveness and grading (without MiB-1) of ductal carcinoma ($p= 0,000 < \alpha= 0,05$). Variable of protein c-erbB2 is expressed by the most tumor and followed by protein p53, MIB-1 and pRb in various tumor diameters, cell differentiation, progressiveness and grade (without MIB-1) of ductal carcinoma ($p= 0,000 < \alpha= 0,05$). By identifying the difference of protein expression; expected to complete the available parameter and used as a principle to determine prognosis and proper therapy of patients with breast cancer.

This study suggest that the result of study can to determine prognosis and appropriate therapy in the patients with breast cancer. Author suggest that the result of study can to describe the mechanism of protein expression changes to the progressiveness of breast cancer growth.

ABSTRACT

Protein Expression c-erbB2, p53, pRb and MIB-1 in Breast Cancer

Imam Susilo

Cancer of the breast is one of the most common human neoplasms, accounting for approximately one quarter of all cancers in females. It is associated with the lifestyle, and still occupies the second rank of females cancers morbidity and mortality rate in Indonesia. Early detection and advances in treatment have begun to reduce mortality rates in several countries. Breast cancer is further characterized by a marked genetic susceptibility. Through the use of protein gene expression profile, it may become possible to predict clinical outcome in individual patients. Molecular pathologic approach may affer a prospective promise eventhough the genetic mechanism of molecular carcinogenesis of breast cancer is still unclear.

In this study, we attempt to explain the role of mechanism of c-erbB2, p53, pRb and MIB-1 oncoprotein expression on cancer cells based on molecular pathology science. By using immunohistochemistry examination,it showed cellular activity in carcinogenesis of breast cancer.

The statistic was investigated by using multivariate analysis-design of protein gene expression measured and counted with Olympus microscope BX-50 Japan, from immunohistochemistry processing with monoclonal antibody-to identify the differences of protein gene expression in breast cancer with $\leq$ 2 cm, > 2 - $\leq$ 5 cm, and > 5 cm diameters; the differences of protein gene expression in breast cancer with well, intermediately and poorly differentiated cell tumors; the differences of protein gene expression in breast cancer with insitu, invasive and metastatic progression, and the differences of protein expression in low, intermediate and high grade tumors

The result designated that there was a significant difference among four breast cancer groups ($p = 0{,}000 < \alpha = 0{,}05$) and all oncoprotein expression (without MIB-1) contributed on cellular activity in carcinogenesis of breast cancer. The most significant prognostic indicator among these biological parameters were differentiated cell tumor and protein gene expression (c-erbB2 and p53) It showed that malignancy occurred in genetic lesion accumulation.

Key words: Breast cancer ; Prognosis ; Molecular pathological role (c-erbB2, p53, pRb and MIB-1 protein expression)