



UNIVERSITI PUTRA MALAYSIA

**PROGNOSTIC MARKERS OF RESISTANCE AND RELAPSE IN
ACUTE LEUKAEMIA**

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LEUKAEMIA**

By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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Specially dedicated to,

Allah, Most Gracious, Most Merciful

Thank you for the knowledge, the sustenance and my family, my children, husband, mother, brother and sisters for their love, understanding, encouragement and patience.

May Allah bless you all.



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Leukaemia is the malignant transformation of cells of the haemopoietic system. It is the most common cancer in children. The Ministry of Health, Malaysia (1999) reported an incidence rate of 3.36 in every 100,000. Nevertheless, leukaemia is nine times more frequent in adults. It is differentiated into acute and chronic leukaemia by morphology of the cell. Acute leukaemia is also a more aggressive disease. Chronic leukaemia is rare among children. The majority of leukaemia (83%) is acute leukaemia (National Cancer Registry, Malaysia, 2002). The two main cell types are the lymphoid and myeloid lineage.

The conventional method for the treatment of acute leukaemia is chemotherapy. Children achieve a remission rate of > 90%. In adult acute lymphoblastic leukaemia (ALL) remission is only 65-80%. Response rate is worst among adults with acute myeloid leukaemia (AML), 70% in young adults decreasing to 25% in the elderly. The rest are resistant to treatment. Many patients relapse within the first two years after



achieving remission. Children achieve a cure rate of 57-73% while adults have a dismal 35%. The relapsed disease is usually resistant to chemotherapy.

Many factors have been implicated in the cause of resistance and relapse. Much work is still needed to explain the mechanism involved to improve treatment and find alternative targets for therapy.

We postulate that the cause of resistance and relapse arises from the biology of the cell and its response upon exposure to chemotherapeutic drugs. We collected *de novo* acute leukaemia samples to determine the phenotype and survival potential of the cells and obtained samples from patients undergoing induction therapy to observe for changes with regards to inhibition of survival pathways and the response of the apoptotic machinery. We also collected resistant and relapsed samples to analyze for these factors. Furthermore, we cultured primary acute leukaemia cells to observe the behaviour of the cells *in vitro*.

We found resistant and early relapsed samples had a more immature phenotype being of the French-American-British (FAB) M1, M4 and M5a subtypes. We used MTT assay to measure proliferation, and showed high proliferative potential among these samples, reflecting self-renewal capacity and a stem cell nature. We obtained a significant difference between the proliferative potential of cells from patients with longer remission duration compared to patients with shorter survival period ($p=0.013$). Very few reports have used this technique to correlate with treatment outcome. We report the first significant correlation between lower proliferative potential and long term clinical outcome.

We were also able to show a significantly ($p=0.033$) higher rate of proliferation in the earlier B-cell ALL subtype (null ALL, CD10-) compared to the more mature (pre-B ALL, CD10+) subtype. Thus, we determined a new way to recognize the distinction between these two groups. In ALL cases, we found samples with a higher S-phase fraction were associated with a younger age group ($p=0.000$) and better survival. This was also not reported before.

We observed resistant and relapsed samples also expressed more growth factors such as c-kit receptor, IL (Interleukin)- 1β , GM-CSF (granulocyte-monocyte colony stimulating factor) and IL-18 and this corresponded with higher levels of pro-survival factors such as Bcl-2 and phosphorylated Bad. We found relapsed samples to have a higher expression of the multi-drug resistance genes especially MRP1 and also MDR1 and LRP.

We report the first few observations of cells treated *in vivo*. We found resistant samples maintained high levels and increased levels of growth factors. This was supported by increased phosphorylation of signaling mediators such as Akt, p42/44, transcription factors such as FKHR (Forkhead) and sequestration of pro-apoptotic genes such as Bad. In cells that responded to treatment, down-regulation of these factors occurred and at the same time up-regulation of factors involved in pathways leading to cell death, such as TNF- α , p38 and Jnk was observed. The Fas receptor/ligand system did not appear to mediate chemotherapeutic induced death. The decoy receptor was also not involved in resistance. DR5 was also observed up-regulated in cells responding to chemotherapy. DR4 may play a role in resistance.

We found many changes occurred when cells were cultured including acquisition of mature markers, up-regulation of growth factors and corresponding signaling mediators. However, changes that alter a prognostic factor, e.g. an increase in S-phase fraction, render the culture no longer representative of *in vivo* treatment. Nevertheless, we found cell culture can still provide information that cannot be obtained *in vivo* e.g. by removing cells from the inhibitory factors of the original environment revealed novel insights that may be utilized in improving treatment.

Thus, many factors may play a role in causing resistance and relapse in acute leukaemia. A comprehensive and more thorough examination of each sample may be required to better understand the mechanism behind it. Furthermore, there is a need for continuity with the present samples for future techniques and other factors of study.

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MARKER PROGNOSTIK RESISTAN DAN RELAPS DALAM LEUKEMIA AKUT

Oleh

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Leukemia adalah pertumbuhan abnormal sel sel yang membentuk sel darah. Ia merupai barah paling kerap dijumpai dikalangan kanak-kanak. Kementerian Kesihatan (1999) melapor kejadian seramai 3.36 dalam setiap 100,000. Namun demikian, ia sembilan kali lebih kerap dikalangan dewasa. Leukemia boleh dibezakan kepada leukemia akut dan leukemia kronik berdasarkan rupa bentuk (morfologi) selnya. Leukemia akut juga merupai penyakit yang lebih agresif. Leukemia kronik jarang dijumpai pada kanak-kanak. Kebanyakkan leukaemia (83%) ialah jenis leukaemia akut (National Cancer Registry Malaysia, 2002). Dua jenis sel yang utama ialah limfoid and myeloid.

Cara rawatan yang utama untuk akut leukemia ialah kimoterapi. Lebih daripada 90% kanak-kanak dapat diubati dengan cara ini. Dikalangan dewasa, 65-80% daripada sel pesakit leukemia akut limfoid dapat dihapuskan dengan cara pengubatan ini. Untuk leukemia akut myeloid pula, kadar pesakit yang dapat diubati turun dari 70% antara yang muda kepada 25% antara yang tua. Pesakit selebihnya tidak dapat diubati kerana sel barah tidak mati (apoptosis) dengan kimoterapi. Sel pesakit ini dikatakan resistan



terhadap kimoterapi. Pada ramai pesakit yang pada mulanya sembuh, penyakit ini akan timbul kembali (relaps). Peratus kanak-kanak yang tidak mengalami relaps ialah 57-73% dan antara dewasa hanya 35%. Sel leukemia relaps biasanya resistan terhadap kimoterapi.

Banyak faktor yang mungkin terlibat dalam membentuk sel yang resistan dan relaps. Kajian-kajian perlu dilakukan untuk mengenalpasti mekanisma yang terlibat supaya cara pengubatan dapat diperbaiki dan target baru pengubatan dapat dicari.

Kami berpostulasi bahawa kejadian resistan dan relaps adalah disebabkan sifat semulajadi sel tersebut yang menyebabkannya dapat melawan kesan kimoterapi. Maka sampel-sampel akut leukemia baru dikutip dan ditentukan fenotipnya dan keupayaan sel untuk hidup. Untuk menganalisa perubahan yang berlaku ketika sel dikenakan kimoterapi, sampel diambil dari pesakit yang sedang diinduksi. Sampel dari pesakit yang resistan dan relaps juga diperolehi untuk dikaji faktor-faktor yang sama. Selain itu, sel leukemia akut juga di kultur untuk mempelajari kelakuannya dalam kultur.

Hasil kajian kami menunjukkan sel resistan mempunyai sifat sel baru terbentuk (immature). Ini dapat dilihat dari pengelasan FAB (French-American-British) yang kebanyakannya M1, M4 dan M5a. Potensi menambah bilangan dengan banyaknya menbayangkan sifat “immature” nya. Kami mengguna esei MTT untuk memerhati pertumbuhan sel dan mendapati banyak sel resistan mempunyai kadar pertumbuhan yang tinggi. Lebih-lebih lagi, kami mendapati perbezaan yang signifikan ($p=0.013$) dalam kadar pertumbuhan sel antara pesakit yang relaps awal dengan yang dapat

bertahan lama dari mendapat penyakit itu semula. Cara mengesei ini jarang dipakai dan kami melapurkan pemerhatian signifikan pertama ke atas yang tersebut di atas.

Kami juga dapat menunjukkan perbezaan signifikan ($p=0.033$) antara kadar pertumbuhan sel B limfosit yang lebih muda (null, CD10-) dengan yang lebih matang (pre-B, CD10+) dan dengan demikian menentukan cara baru membeza antara dua kumpulan sel ini. Kami juga mendapati bahawa untuk leukemia akut jenis sel B, bahagian fasa S nya adalah lebih tinggi dikalangan pesakit yang lebih muda berbanding yang lebih tua ($p=0.000$). Ini belum pernah di tentukan.

Sampel resistan dan relaps juga didapati mengexpresi gen faktor pertumbuhan (growth factor) seperti reseptor c-kit, IL (Interleukin)-1 β , GM-CSF (granulocyte-monocyte colony stimulating factor) dan IL-18 dengan lebih banyak daripada sampel sel yang diperolehi dari pesakit yang sensitif kepada kimoterapi. Ini juga diiringi peninggian dalam expresi faktor “pro-survival” seperti Bcl-2 dan fosforilasi protin Bad. Sampel relaps juga didapati menghasilkan banyak gen “multi-drug resistance” seperti MRP1, MDR1 dan LRP.

Kami melapurkan pemerhatian pertama ke atas sel yang telah diberi kimoterapi. Sel dari sampel resistan yang dikenakan kimoterapi mengekalkan paras tinggi faktor pertumbuhan dan meningkatkan fosforilasi protin perantara seperti Akt, p42/44, “transcription factor” FKHR (Forkhead), dan Bad, yang kesemuanya mengutuskan isyarat untuk sel terus hidup. Pada sel yang sensitif terhadap kimoterapi, protin perantara seperti p38 and Jnk pula di fosforilasikan untuk mengutuskan isyarat “apoptosis”. Kami mendapati, DR (death receptor)-4 dan mungkin TNF (Tumour

necrosis factor)- α terlibat dalam proses “apoptosis”. DR4 pula mungkin memainkan peranan dalam resistan.

Kami mendapati banyak ciri-ciri sel telah berubah apabila sel dikulturkan termasuk pemilikan ciri-ciri kematangan sel, kenaikan faktor pertumbuhan sel dan protin perantaraan. Perubahan yang mengubah “prognostic factor” sesuatu sample e.g. peningkatan dalam peratus fasa-S akan menjadikannya tidak sesuai untuk dibandingkan dengan kajian pangubatan *in vivo*. Walaubagaimanapun, kami perhatikan kerja-kerja kultur dapat menyumbang kepada pengetahuan baru tentang sel dan persekitaran asalnya yang mungkin mengandungi faktor “inhibitori” yang menyekat perkembangannya seperti sel biasa. Pengetahuan ini dapat membantu dalam “mengembalikan” sel kanser ini kepada sel normal yang dapat di hapuskan dari tubuh dengan semulajadinya.

Maka banyak faktor yang menyebabkan sel resistan dan relaps dalam leukemia akut. Pemeriksaan yang lebih komprehensif dan mendalam mungkin diperlukan atas setiap sampel untuk memahami mekanisma yang terlibat. Maka perlunya penyinambungan dengan teknik atau faktor baru atas sampel sedia ada.

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