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我々はゲフィチニブの副作用出現を予測する方法を開発するため、大規模なSNP解析を行った。対象は16週間以上にわたりゲフィチニブの投与が行われた肺非小細胞癌患者42名である。副作用の有無は7日ごとに確認しNCI-CTCグレードによって評価した。加えて治療28日目にゲフィチニブの血中濃度を測定した。また、これらの患者の末梢血よりDNAを採取し、512個の遺伝子をカバーする3,717個のSNPのジェノタイピングを行った。治療期間中、42名中23名がグレード1以上の下痢を示した。SNP解析の結果、7個のSNPsが下痢の出現と極めて高い相関を示した(p=0.000025)。これらのSNPは同じ遺伝子（GENE1）内に存在し、3個はプロモーター領域、残りはイントロン内に存在していた。また、別の遺伝子(GENE2)内に存在する2個のSNPはゲフィチニブの血中濃度との相関を認めた(p=0.00056)。これら2つの遺伝子は同じ遺伝子ファミリーに属しており、ゲフィチニブの代謝にこれらのファミリーが関与することが示唆された。これらの結果を用いれば高い精度で副作用出現を予測することが可能となる。

Keyword: gefitinib, SNP

P-1354 *CYP2C8*遺伝子プロモーター領域の多型解析

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Genetic polymorphisms in the *CYP2C8* promoter region

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CYP2C8はタキサン系抗癌剤のなかでPaclitaxel（TXL）に特徴的な代謝酵素とされ，同剤に対する個体の応答を考える上で極めて重要な因子である。しかしながら，その遺伝子多型の存在や意義に関してはいまだ不明な点が多い。TXL応答予測への展開を念頭に，ヒト培養腫瘍細胞36株の*CYP2C8*遺伝子プロモーター領域の塩基配列を解析した。その結果，既知の-271，-370 position（ATGを+1として）に加え，-411 positionに新たな多型を見出した。頻度は-271C/C: 86.1%，C/A: 11.1，A/A: 2.8，-370T/T: 51.5，T/G: 21.2，G/G: 27.3，-411T/T: 51.6，T/C: 25.8，C/C: 22.6であった。遺伝子型と遺伝子発現量とを比較すると，-370Gアリルを持つ細胞は発現量が低く，-411Cアリルを持つ細胞は発現量が高い傾向にあり，*CYP2C8*プロモーターレポーター実験では，-411Tに比し-411Cでは有意にプロモーター活性が高い可能性が示された。現在、臨床検体におけるこれら遺伝子多型の意義について検討を進めている。

Keyword: CYP, SNP

P-1355 緑膿菌体外毒素による細胞毒性の遺伝子発現解析

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Gene expression profiles in the cellular response to recombinant *Pseudomonas* exotoxin A

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Pseudomonas exotoxin A (PE) is a single-chain toxin secreted by *Pseudomonas* aeruginosa. It kills cells by catalyzing the irreversible ADP-ribosylation and subsequent inactivation of elongation factor2. PE consists of three major domains termed Ia, II, and III. Domain Ib is a minor domain. Domain Ia has been replaced by a single-chain antigen binding protein (including growth factors or single chain antibodies) to produce targeting toxin for cancer therapy. Some of PE derived toxins are now tested under the clinical trials for the targeted cancer therapy. The PE-related toxicities are capillary leak syndrome, renal and hepatic injury mediated by Kupffer cell-secreted TNF-α. In this study we examined gene expression profiles in primarily cultured human renal tubular cells after an exposure to PE using HG-U133 set (Affymetrix) to clarify molecular mechanisms of renal damage. We found significant changes in transcription of enzymes related to amino acid synthesis and RNA binding proteins.

Keyword: Toxin, Immunotoxin

P-1356 選定された指標遺伝子群の発現データを用いる食道癌化学療法効果予測モデル

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Chemosensitivity prediction models in esophageal cancer using expression data of selected marker genes.

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【目的】食道癌における抗癌剤治療の定量的効果予測モデルを確立する【方法】ヒト培養食道癌細胞20株を用い、1)cDNA microarray解析とMTT assayにより遺伝子発現量とIC_{50}値を求め、その順位相関解析から5-FU、CDDPなど8種類の抗癌剤の効果に関連する遺伝子を抽出、2)その中から機能の証明された遺伝子を選定、3)それらの発現量を多変量解析によりIC_{50}値に変換する効果予測式を作成した。【結果】食道癌細胞において抽出された効果関連遺伝子は他の腫瘍細胞系のそれらと著明に異なり、他の細胞系で設定された効果予測式を食道癌へ直接転用することは困難と考えられた。食道癌における有力な効果予測指標として13遺伝子が抽出されたが、そのうち*E2F1*、*ERCC2*、*GCLC*など8遺伝子は食道癌に特異的であった。これら13遺伝子に関し、その発現量と抗癌剤効果との関連性がreal-time RT-PCR定量的発現解析でも追認されたことから、多変量回帰分析によって、それら13遺伝子の発現データの代入により各抗癌剤の効果(IC_{50}値)を予測し得る計算モデルを試作した。現在その有用性評価を行っている。

Keyword: Personalized medicine, Chemosensitivity prediction

P-1357 日本人大腸癌におけるTS遺伝子3' UTRの6bp deletion polymorphismとTS発現

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Polymorphisms within 3UTR of the thymidylate synthase gene and TS expression in Japanese colorectal cancer patients

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【目的】TS遺伝子の3'-untranslateed region (3'UTR)には－6bp/1494 deletion polymorphismが存在し（Cancer Epidemiol Biomarkers Prev 2000)、－6bp alleleではmRNA instabilityをきたすことが報告されている（Pharmacogenetics 2004)。今回、日本人大腸癌における－6bp/1494 deletion polymorphismの頻度と腫瘍内TS発現との関係、補助化学療法におけるその意義を検討した。【対象と方法】大腸癌255症例（stage 1-3)。正常組織DNAにて6bp deletion多型をPCR-RFLPで解析した。原発巣のTS発現はELISAで測定した(ng/mg protein)。生存解析は5-FU系補助化学療法が試行された128例で行った。【結果】－6bp deletion多型の頻度は－6/－6(115例 45%)、－6/＋6(110例 43%)、＋6/＋6(30例 12%)。TS発現は－6/－6: 49.6±41.2、－6/＋6: 56.4±48.2、＋6/＋6: 62.2±61.3 (P=0.76, Kruskal-Wallis test)。補助化学療法群での5年健存率は－6/－6 (56例): 81%、－6/＋6および＋6/＋6(67例): 79% (P=0.90)。【総括】TS 3' UTRの6bp deletion polymorphismと腫瘍内TS発現に有意な関連は認めない。また、5-FU系補助化学療法のefficacious markerではない。

Keyword: Pharmacogenomics, Thymidylate synthase

P-1358 Cancer Cell Informaticsによる毒性物質の評価

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Evaluation of toxic substances by Cancer Cell Informatics

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Cancer Cell Informaticsとは、39種ヒト癌細胞株パネル（JFCR-39）による感受性試験とインフォーマティクスとをリンクしたメカニズムオリエンテドな化合物評価法である。我々は、本法を確立し、抗癌物質の

response/>control mean+1SE) was 63.6% (7/11) and the negative predictive value (the number of patients who failed to obtain the response/<control mean+1SE) was 70.6% (12/17). The sIL2R levels on both day 7 and 60 did not show any difference between the groups. Overall, these findings demonstrate that serum sIL2R levels increase in patients with aplastic anemia. Pretreatment serum levels of sIL2R might be closely related to clinical outcome following IS therapy, reflecting the immunological abnormalities in individual patients.

Abstract# 4220

CML Developed in a Japanese Family Transmitting a Novel Point Mutation in the Thrombopoietin Gene(TPO). Yasuo Oshima,[1,2] Norio Komatsu,[2] Keiya Ozawa,[2] Akio Fujimura*.[1] *[1]Clin Pharmacol, Jichi Med Schl, Minamikawach, Kawach, Japan; [2]Hematol, Jichi Med Schl, Minamikawach, Kawach, Japan.*

Introduction: Four families are reported to have hereditary thrombocythemia (HT) with a mutation in TPO. Their clinical manifestation is essentially thrombocytosis without leukemia. CML is one of myeloproliferative disorders, and shows leukocytosis and thrombocytosis associated with a proliferation of malignant clone originated from a hematopoietic stem cell (HSC). The incidence of CML is about 5 per 100,000 in Japan. Mutations of cytokine receptor including c-kit, flt-3 and G-CSF receptor are reported as a cause of AML. Especially flt-3 abnormalities are found in about 20% of AML. However, abnormality of c-mpl or TPO is not reported as a cause of leukemia. In this paper, we analyzed a CML case with novel point mutation in the TPO who still had thrombocytosis after cytogenetic complete response.

Case: Japanese, 35 y.o., male, complained leukocytosis. He had a family history of thrombocytosis in 4 individuals over 3 generations. A physical examination revealed a moderate splenomegaly. Laboratory tests at the time of diagnosis were as follows; WBC 141,000/μl (blast 1.8%, promyelo 2.4%, myelo 20.0%, meta 8.2%, stab 24.2%, seg 22.2%, immature eosinophils 1.8%, eosinophil 3.6%, immature basophils 0.4%, basophils 10.4%, mono 1.0%, lymphocytes 4.0%, erythroblast 3%), PLT 641,000/μl and NAP score 53 (nl; 156-271). Bone marrow showed hypercellularity with the increased megakaryocytes(Meg), bcr-abl fusion mRNA positive, Ph1 chromosome positive. After 5 months treatment with STI571, most of clinical findings including karyotype and fusion mRNA turned to be normal, but thrombocyte(PLT) still showed more than 1,000,000/μl. At this time, serum TPO concentration was 8.14 f mole/ml (nl; 0.40 +/- 0.28 f mole/ml, mean +/- SD). Genetic analysis of TPO revealed novel point mutation at splicing donor site of 3'-end of the exon3. A point mutation at splicing donor site is reported to cause an exon-skipping and intron-retention, which induce a malfunction of a suppressive post-transcriptional and translational regulation, and consequent high-level expression of functional TPO protein.

Discussion: TPO was cloned as a c-mpl ligand, which leads to the production of PLTs. Its receptor is a c-mpl proto-oncogene product, which is expressed not only in Meg, but also in HSC. Thus, TPO can stimulate HSC. The c-mpl transgenic mice are reported to have the increased Meg, its committed progenitor and PLT. Knockout mice of TPO presented not only the decreased Meg, but also multi-lineage committed progenitors. Thus, a modulation of c-mpl or its ligand function affects on both Meg and HSC. The c-mpl was cloned as a cellular homolog of a viral oncogene, v-mpl of myeloproliferative leukemia virus (MPL). The MPL causes myeloproliferative leukemia syndrome through v-mpl function in mice. Since v-mpl and c-mpl indicate high homology, it is possible that abnormal c-mpl function causes v-mpl like response. Through continuous stimulation of c-mpl signal, high TPO concentration may have induced a malignant transformation of HSC or supported a survival of an immature malignant clone in the present case. Improvement of thrombocytosis in CML is one of hematological responses to an anti-CML treatment such as STI571. In such a case who had good response other than thrombocytosis, an existence of HT might be considered. On the other hand, during following up HT family, occurrence of CML should be noted.

Abstract# 4221

Erythropoietin Did Not Activate the JAK/STAT or the ERK1/2 Pathway in the Human Colorectal Cell Line, HT-29. Jennifer Tullai*, Carmelata Chitikila*, Ze'ev Gechtman*, Xiwei Wang*, Linda Jolliffe*, Francis Farrell*. (Intr. by Linda Mulcahy) *Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, LLC, Raritan, NJ, USA.*

Erythropoietin (EPO) is the cytokine essential for erythropoiesis; however, the expression of EPO and EPO receptor (EPOR) is not limited to cells of erythroid lineage. Erythropoietin and EPOR expression has been localized to numerous non-hematopoietic cells and tissues including endothelial, neuronal and ovarian. Moreover, several tumor types have been shown to express both EPO and EPOR and display increased expression upon hypoxia. To shed light on the potential biological role of EPOR and tumor cells we characterized the response of human colon carcinoma HT-29 cells to EPO. Functionality of the erythropoietin receptor was assessed by radio-labeled ligand binding, cellular proliferation/signaling, and gene expression using DNA microarrays. Receptor binding experiments using [^{125}I]-EPO did not reveal measurable EPO binding activity present on the surface of HT-29 cells under both normoxic and hypoxic conditions. Moreover, EPO failed to induce cellular proliferation or an increase in the phosphorylation state of STAT5, EPOR or ERK1/2 under normoxic or hypoxic conditions at supra-pharmacological levels (25 IU/ml). Gene expression analysis revealed no significant change in gene expression in response to EPO (5 IU/ml) under normoxic conditions. On the other hand, over 347 genes exhibited greater than a 1.5 fold change in gene expression when cells were cultured under hypoxic conditions (1 % O_2). When EPO was administered to cells in the hypoxic state, 36 additional genes were observed (9 and 27 up-regulated or down-regulated, respectively). That HT-29 cells exhibit minor transcriptional changes in response to EPO raises the possibility that EPO may signal in HT-29 cells. However, the mechanism for this response is not through the previously described EPO/EPOR signal transduction pathway. This conclusion is supported by the apparent lack of EPO receptor expression on the cell surface. These results suggest that tumor microenvironment, e.g., hypoxia, exerts a greater effect than that seen by exposure to erythropoietin.

Abstract# 4222

Early Results Suggest That Epoetin Alfa 60,000 U Every 2 Weeks Improves Hemoglobin in Patients with Cancer Not Receiving Chemotherapy or Radiotherapy. Daniel Shasha*, David H. Henry*, Denise Williams. *Phillips Ambulatory Care Center, Beth Israel Medical Center, New York, NY, USA; Joan Karnell Cancer Center, Pennsylvania Hospital, Philadelphia, PA, USA; Therapeutic Area Head, Oncology, Ortho Biotech Clinical Affairs, LLC, Bridgewater, NJ, USA.*

Epoetin alfa has been shown to increase hemoglobin (Hb), decrease transfusion requirements, and improve quality of life (QOL) in patients receiving chemotherapy (CT) and/or radiotherapy (RT). However, the efficacy of epoetin alfa in cancer patients receiving neither CT nor RT is not as well characterized, particularly at doses less frequent than 3 times weekly. The objective of this open-label, nonrandomized, multicenter pilot study was to evaluate epoetin alfa 60,000 U subcutaneously (SC) every 2 weeks (Q2W) in 50 anemic patients with cancer who were not receiving CT or RT. The primary efficacy endpoint is the proportion of patients achieving a hematopoietic response (HR; Hb increase ≥2 g/dL from baseline and/or Hb increase to ≥12 g/dL at any scheduled visit) independent of transfusion within 28 days. Patients with histologically confirmed nonmyeloid malignancy, Hb ≤11 g/dL, and who had not received CT in the previous 8 weeks or RT in the previous 4 weeks were enrolled. Patients were permitted to receive hormonal therapy, androgen deprivation therapy, and/or immunotherapy. Patients were to receive epoetin alfa 60,000 U SC Q2W, with escalation to 80,000 U Q2W after 4 weeks if Hb increased ≤1 g/dL. Dose will be titrated to maintain Hb ≤13 g/dL; all patients were to receive oral ferrous sulfate 325 mg daily. Patients will be treated for up to 12 weeks, with a 4-week follow-up after last dose of study drug. As of July 2004, 18 patients are evaluable for efficacy (modified intent-to-treat, ie, all enrolled patients who received ≥1 dose of study drug and had ≥1 postbaseline Hb or transfusion evaluation) and 18 for safety (all enrolled patients who received ≥1 dose of study drug). Mean age was 74.4 ± 8.7 yrs, mean baseline Hb was 10.0 ± 1.0 g/dL (n=18), and 7/18 were men. Of these patients, 7 completed 4 weeks, 3 completed 8 weeks, and 1 completed 12 weeks on study. The HR of these patients was assessed regardless of how many weeks of study they completed at the time of this interim analysis; HR was 44.4%. Hb increased 1.4 ± 0.7 g/dL (n=11) after 4 weeks and 1.2 ± 1.4 g/dL (n=4) after 8 weeks of treatment. Adverse events were limited to 1 patient, who experienced back pain and epistaxis. No patients died during the study, and no thrombotic vascular events were reported. Early results of this study suggest that epoetin alfa 60,000–80,000 U SC given every other week is well tolerated and appears to effectively increase Hb in anemic cancer patients not receiving CT or RT. The study is currently ongoing.

Abstract# 4223

Epoetin Alfa 40,000 U QW Increases Hb and Is Safe in Anemic Patients with Cancer Not Receiving Chemotherapy or Radiation Therapy. Daniel Shasha*, Denise Williams. *Phillips Ambulatory Care Center, Beth Israel Medical Center, New York, NY, USA; Therapeutic Area Head, Oncology, Ortho Biotech Clinical Affairs, LLC, Bridgewater, NJ, USA.*

Patients (pts) with cancer often become anemic as a result of the disease and its treatment. Epoetin alfa (EPO) administered 150 U/kg SC 3 times a week in pts with cancer-related anemia not receiving chemotherapy (CT) or radiation therapy (RT) significantly reduces transfusion requirements, increases hemoglobin (Hb), and improves quality of life (QOL). EPO 40,000 U SC QW is effective in pts with cancer-related anemia receiving CT +/- RT and may be a treatment option for pts not receiving CT or RT. This open-label, multicenter, pilot study investigated clinical outcomes and safety of EPO 40,000 U SC QW in anemic (Hb <11 g/dL) pts with cancer not receiving CT or RT. Treatment duration was up to 12 wks with a 4-wk posttreatment observation period. If Hb increased <1 g/dL after 4 wks, dose was increased to 60,000 U SC QW. EPO dose was reduced for Hb >15 g/dL; dose reduction was considered for Hb increase >1.3 g/dL over 2 wks in the original protocol. Primary endpoint was proportion of pts achieving a ≥1-g/dL or ≥2-g/dL increase in Hb from baseline (BL; independent of transfusion within the previous 28 days) at any time during the study. Secondary endpoints included transfusion requirements and QOL (measured with the Linear Analog Scale Assessment; LASA). The study was temporarily suspended due to concerns of a potential increased risk of thrombotic events if Hb >13 g/dL, and restarted with an upper Hb limit of 13 g/dL and rate of rise of Hb of 1 g/dL over any consecutive 2-wk period, independent of transfusion. Due to the interruption in therapy, 3 efficacy populations were evaluated: pts who had a post BL Hb value or transfusion (modified intent-to-treat [MITT], n=91); pts who completed the study prior to suspension (presuspension, n=37); and pts who entered the study, had treatment suspended, and completed after the study was restarted (suspension, n=33). Ninety-five pts were evaluable for safety: mean age, 69 years; 45% women; 85% ECOG 0-1; mean BL Hb 10.4 ± 0.73 g/dL. For the 3 populations analyzed, 73/91 (80%), 33/37 (89%), and 29/33 (88%) pts had an Hb increase ≥2 g/dL for the MITT, presuspension, and suspension populations, respectively. 13/91 (14%), 2/37 (5%), and 3/33 (9%) pts had at best an Hb increase ≥1 g/dL for the MITT, presuspension, and suspension populations, respectively. For the MITT population, mean change in Hb from BL after 12 wks was 2.9 ± 1.54 g/dL. Mean Hb decreased 1.4 ± 1.10 g/dL during the posttreatment observation period. One pt was transfused on study. Both wk 9 and wk 17 LASA scores increased significantly from BL in all categories (Energy Level, Daily Activities, and Overall QOL; $P<0.001$). EPO dose was increased in 15 (16%) pts and decreased or held in 61 (64%) pts during the study. Mean time to first dose reduction or hold was 38 days. Most commonly reported AEs were fatigue (19%) and nausea (13%). Twenty-four (25%) pts had ≥1 serious AE. Four (4%) pts discontinued due to an AE. One pt died on study due to disease

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Primary Cell Preparation of Human Renal Tubular Cells for Transcriptome Analysis

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We initiated a toxicogenomics project using Affymetrix GeneChip® HG-U133A and HG-U133B arrays harboring 45,000 probe sets representing more than 39,000 transcripts to analyze gene expression in primary cultures of human cells after exposure to chemicals that cause tissue toxicity. In order to assess the quality of the samples studied, we prepared primary human renal cortical cell cultures from surgically resected human kidney and evaluated the origin of the cells and the effects of cryopreservation. We analyzed the primary cultures using GeneChip and compared their expression patterns with those in the Novartis Research Foundation (GNF) Gene Expression Database. The comparison with the GNF database revealed that the gene expression pattern of the cultured cells was compatible with kidney cells, indicating that we had purified human renal cortical cells. Due to the purification procedure, the primary cultured cells could be a mixture of renal components; however, we identified the major population as renal proximal tubule cells by assessing gamma-GTP activity and Glut2 antigen expression. We compared gene expression in the cells before and after cryopreservation. The expression of 567 selected housekeeping genes was unchanged by cryopreservation (Pearson's correlation coefficient $r = 0.980$; $p < 0.0001$). The analysis of more than 39,000 transcripts after normalization revealed no significant changes in expression. These results indicate that our method is satisfactory for obtaining adequate primary cell cultures of renal origin and that gene expression was not significantly changed by cryopreservation.

Keywords Cryopreservation, GeneChip, Genomics, Primary Culture, Renal Tubular Cell, Transcriptome

Our current assessments of toxicity in the development and regulation of newly created medicines and chemicals rely on simplified assays and models that may underestimate the biological complexity underlying toxic effects. Interfacing genomic technologies with toxicology provides a more profound way to investigate biological complexity and to create a more systematic toxicology. One purpose of our toxicogenomics project is to identify potential (surrogate) biomarkers that can distinguish safe agents from toxic ones in vitro; another purpose is to establish a systematic toxicology that includes more systematic mechanisms, pathways, and hypotheses of toxic events. Our goals are to reduce the time and money required to develop safe, new medicines and chemicals in the future and to improve drug risk assessment.

Since the technology is new, there are no standard technologies, no standard experimental protocols, no robust tools for data analysis, and no knowledge of how transcription products relate to toxicity. Therefore, in late 1999, governments in the US, Europe, and Japan, more than 30 pharmaceutical, chemical, agricultural, and consumer product companies, and several academic institutes (University of Surrey in the UK, Michigan

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State University, and Medical College of Wisconsin) initiated toxicogenomic projects. The program consists of hepatotoxicity, nephrotoxicity, genotoxicity, and database working groups. According to the interim findings of the nephrotoxicity working group, which were presented at an ILSI-HESI meeting on toxicogenomics in risk assessment (June 6, 2003, Fairfax, VA), transcriptional analysis yielded strong topographic specificity and some mechanistic information; the frequency of individual animal transcript changes was reduced in nonresponders and increased in cases of severe toxicity; the use of pooled RNA samples may have a dilution/skewing effect on the interpretation of genetic responses; and microarray technology has a sensitivity at least equal to traditional toxicology endpoints. Their data indicated that responder and non-responder animals exist within the same species; therefore, there must be differences in expression profiles between species. This makes analyses of human gene expression profiles essential for toxicogenomic projects. In that context, we are performing genome-wide gene expression analyses using primary human tissues after exposure to therapeutic agents or other environmental chemicals that may influence human health.

Since the kidney filters and concentrates extrinsic compounds and intrinsic waste metabolites, renal tissues are always exposed to harsh conditions and are often damaged by medicines such as aminoglycosides and platinum compounds, including cisplatin and carboplatin. Therefore, we placed a priority on the kidney as a target organ in our project. There are conventional methods for purifying human renal tubular cells for biomedical research (Detrisac et al. 1983, 1984; Nagineni et al. 1984; Horster and Stopp 1986), and since our study depends on mRNA expression profiling, it is essential to confirm cell characteristics using mRNA expression.

One obstacle to the study of primary cultured human cells is the limited availability of freshly isolated human tissues. Commercial suppliers of primary cultured human tissues usually provide cells cryopreserved in a deep freezer or liquid nitrogen. Even if freshly isolated tissues are available, cryopreserved cells that can generate experimental outcomes that represent meaningful biological responses should improve handling and allow experiments to be repeated to confirm results. There are no published reports on systematic gene expression analyses of the effects of cryopreservation on human primary cultured renal tissues; there are only a few reports on the effects of cryopreservation on animal renal tubular cells (Jung et al. 2001; Kouwenhoven et al. 2001) and human whole kidneys (Salahudeen et al. 2000), and these look at the expression of only a few genes. In this study, we confirmed the origin of cultured renal cortical cells and analyzed the effects of cryopreservation on systematic gene expression in primary cultured renal cells.

MATERIALS AND METHODS

Samples and Purification of Renal Cortical Cells

Patients 18 years of age or older admitted to the Jichi Medical School Hospital for renal resection due to a confirmed solitary tumor in one kidney, the renal pelvis, or the ureter were eligible for the study. Patients with renal dysfunction (i.e., serum creatinine levels greater than 2.0 mg/ml or abnormalities in serum sodium, potassium, or chloride) were disqualified. The patient characteristics are summarized in Table 1. The study protocol was approved by the institutional review boards, including the Bioethics Committee of Jichi Medical School and the Bioethics Committee for Human Gene Analysis. All patients or their legally authorized representatives gave written informed consent before enrollment. The study was designed, conducted, and analyzed independent of any pharmaceutical companies.

When a kidney was resected, the largest part of the tissue, including the tumor, was used for pathological diagnosis; the remaining tissue was regarded as normal cortex and was placed in cold EuroCollins solution (Kobayashi Pharmaceuticals Co., Ltd., Tokyo, Japan), which is routinely used to preserve whole human kidneys for organ transplantation in our institute. A few grams of the tissue were chopped up and washed with EuroCollins solution (Collins et al. 1969). After 60 min of continuous agitation in an intracellular-like solution (Nagineni et al. 1984) with 1500 U/ml dispase (Godo Shusei Co., Ltd., Tokyo, Japan) and 1 mM calcium chloride, cells were incubated in Hank's Balanced Salt Solution (HBSS) (Invitrogen, Carlsbad, CA) with 0.05% trypsin and 0.53 mM sodium ethylenediamine tetraacetate (EDTA) at room temperature until tubule-like debris was seen under a low-power microscope; this usually took 30 to 60 min. The cells were washed and incubated overnight in the Nutrient Mixture F-12 (D-MEM:F-12, 1:1) (Invitrogen) and 10% fetal bovine serum (FBS). Subsequently, the cells were cultured in Williams' Media E (Invitrogen) with 10% FBS at 37°C in 5% CO_2 until used for assays.

Cryopreservation

When the cells reached confluence, they were harvested and mixed with Cell Banker (Juji Field, Inc., Tokyo, Japan). Cells were aliquoted into 2.0-ml cryotubes (Asahi Techno Glass Co. Ltd., Chiba, Japan) and stored in a Nalgene Cryo Container (Nalgene Nunc International K.K., Tokyo, Japan) at −80°C overnight; the frozen cells were then removed from the Cryo Container and stored at −80°C until assayed. The primary cultured cells were grown to confluence, harvested, and used for RNA purification before and after cryopreservation. We compared RNA expression before and after cryopreservation and analyzed the changes.

Gene Expression Analysis

Total RNA was extracted using an RNeasy kit (Qiagen) according to the manufacturer's instructions. Purified RNA products were quantified using a RiboGreen® RNA Quantitation Kit (Molecular Probes, Inc., Eugene, OR) with RNA standards. When the OD_{260}/OD_{280} ratio of RNA was between 1.8 and 2.1, the transcripts were labeled as follows. One microgram of purified RNA was converted to double-stranded complementary DNA (cDNA), which was used to prepare

TABLE 1

Patient characteristics. The characteristics of patients participating in the research project are shown. All laboratory data are within normal range, except the urine blood value in case 5

Patient	Case5	Case6	Case8	Case9	Case10
Age [years old]	57	65	47	76	35
Gender	Male	Female	Male	Male	Male
Warm ischemic time [min]	12	58	38	68	50
Serum urea nitrogen [mg/dl]	13	15	13	18	14
Serum creatinine [mg/dl]	0.86	0.91	0.83	0.78	0.8
Serum sodium [mEq/L]	144	139	140	140	140
Seryn potassium [mEq/L]	4.1	3.9	4.1	4.1	4.1
Serum cloride [mEq/L]	106	102	102	105	101
Urine protein	—	—	—	—	—
Urine glucose	—	—	—	—	—
Urine blood	±	—	—	—	—
Clinical diagnosis	Left renal tumor	Right renal tumor	Left renal tumor	Right renal tumor	Right renal tumor
Histopathology	Nodular carcinoma	Clear cell carcinoma	Clear cell carcinoma	Clear cell carcinoma	Clear cell carcinoma

biotin-labeled complementary RNA (cRNA) for hybridization with the GeneChip test3, HG-U133A, and HG-U133B chips (Affymetrix, Santa Clara, CA), which harbor 45,000 oligonucleotide probe sets corresponding to approximately 39,000 transcripts. Hybridization, washing, and detection of the signals on the arrays were performed with the GeneChip system (Affymetrix) (Oshima et al. 2003). As quality control measures, we analyzed B2-oligo performance, the existence of hybridization control, background noise values, and the 3′/5′ ratio of GAPDH and actin transcripts. If one or more quality tests gave values outside of the acceptable quality range, the data were excluded from the analysis.

Selection of Kidney-Specific Genes and Housekeeping Genes

We used the Gene Expression Database of the Genomic Institute of the Novartis Research Foundation (GNF) to find kidney-specific genes (URL http://web.gnf.org) (Su et al. 2002). First, we calculated the average expression of each gene in all 47 human tissues and human cell lines. Then, we selected genes that are expressed in the kidney at levels at least 10 times the average levels. We excluded genes expressed at levels greater than 3 times the average in any tissue (or tissues) other than kidney. This stringent screening identified eight genes as kidney-specific: KL (205978_at), SLC17A3 (207298_at), AQP2 (206672_at), SLC22A2 (207429_at), KCNJ1 (210403_s_at), TP53BP1 (203050_at), NKCC2 (220281_at), and XPNPEP2 (206484_s_at). NKCC2 expression showed a large variation in the GNF database (data not shown), so we excluded it from the analysis. Since no previous publications support the kidney-specific expression of TP53BP1 or XPNPEP2, we did not use these two genes as kidney-specific positive controls.

We selected 451 housekeeping genes reported earlier (Butte et al. 2001; Hsiao et al. 2001). We used an improved GeneChip (HG-U133) compared with the HG-FL array used by Butte et al. (2001) and consequently converted the data sets from the HG-FL array format into the HG-U133 array format, resulting in 630 probe sets instead of the earlier 451 data sets. This discrepancy is partly derived from the differences in the databases used for the array design (Technical Note, Affymetrix). Since the scanner reads the absence of gene expression with vast variation and minimal reproducibility, we removed approximately 10% of the 630-probe set based on negative expression.

Statistical Analysis

Data analysis was performed using GeneSpring® version 6 software (Silicon Genetics, Redwood, CA) and included normalization, Cross Gene Error Modeling, and parametric tests. For normalization, values less than 0.01 were set to 0.01. All genes in each sample were divided by the median of a list of housekeeping genes. Data were refined using Cross Gene Error Modeling (Rocke and Durbin 2001). First, we calculated the modeled error based on 61 scanned data treated identically. We removed genes with control signals greater than the calculated base/proportion value. The statistical analysis between pre- and post-cryopreserved gene expressions was performed with a non-parametric test and the Benjamini and Hochberg False Discovery Rate for multiple testing.

Cell Staining for Flow Cytometric Analysis

Anti-rabbit goat immunoglobulin labeled with FITC (Immunotech, Cedex, France) was dissolved at 1 mg per 750 μl and mixed with 750 μl glycerol to give a final concentration of 666 μg/ml, the solution was stored at −20°C until used for assays. Cultured cells were harvested after incubation with 0.5% EDTA in phosphate buffered saline at 37°C for 5 min. After the cells were washed with MACS-FACS buffer (2mM EDTA and 3% FBS in phosphate buffered saline), 200 μl MACS-FACS buffer containing 2–5 $\times 10^6$ cells/ml were incubated with 1 μl anti-human Glut2 antibody (H-67, Santa Cruz, Santa Cruz, CA) for 30 min on ice with intermittent agitation. After washing with MACS-FACS buffer, the samples were incubated with 200 μl MACS-FACS buffer containing 2 μl anti-rabbit FITC-conjugated immunoglobulin for 30 min on ice. The cells were washed with ice-cold MACS-FACS buffer, and the cell-bound anti-human Glut2 and FITC-conjugated secondary antibody was determined by BD LSR™ (Becton Dickinson, San Jose, CA) as the fluorescence intensity of FITC. Data collection and statistical analyses were performed with CellQuest software (Becton Dickinson) according to the manufacturer's instructions.

Cell Staining for Gamma-Glutamyl Transpeptidase (gamma-GTP) Activity

We tested gamma-GTP activity because it is a known marker of proximal tubular cells. Cells were cultured on collagen-coated sterile glass slides (Asahi Techno Glass Co. Ltd., Chiba, Japan) at 37°C in a CO_2-incubator for a few days until subconfluent. For gamma-GTP staining, the cells on the chamber glass were fixed with acetone for 2–3 h. After dehydration, the cells were incubated for 5–30 min with staining buffer, which included gamma-glutamyl-4-methoxy-2-naphthylamide and dimethyl sulfoxide, and then with 0.1 N copper sulfate for 2 min. The cells were washed with normal saline and then with water. The stained cells were observed under a microscope.

RESULTS AND DISCUSSION

Purified Renal Cortical Cells

We obtained primary cells from the kidneys of patients in five cases. We tested several isolation methods, (e.g., collagenase or mechanical homogenization) in combination with HBSS containing trypsin and EDTA or HBSS containing trypsin and EDTA alone, but the protocol described in the Materials and Methods section above yielded the best results. Warm ischemia is a concern in studies using viable human samples obtained by surgical operation. To the extent that we tested, we could successfully purify viable cells after up to 68 min of warm ischemia (Table 1).

Next, we identified the origin(s) and characterized the cells. After a few weeks, the cells showed the morphology of uniform epithelial cells, which suggested that the purified cells might have a uniform origin and characters (Fig. 1a). Expression analysis revealed that five kidney-specific genes were present in these cells (Table 2). One of these genes was KL (205978_at). Kuro-o et al. (1997) cloned the mouse KL (*klotho*) gene from a transgenic mouse model with several age-related disorders; the *klotho* gene is named for the Fate in Greek mythology who spins the thread of life. Human KL cDNA clones were isolated by screening human kidney cDNA libraries with a mouse KL cDNA fragment at a low stringency (Matsumura et al. 1998). Interestingly, patients with chronic renal failure (CRF) develop multiple complications reminiscent of the phenotype observed in KL mutant mice. RNase protection, immunoblots, and immunohistochemical analyses demonstrated that KL mRNA expression and protein production were severely reduced in the kidneys of CRF patients (Koh et al. 2001). Koh et al. (2001) proposed that decreased KL expression might be one of the factors underlying the degenerative processes (e.g., arteriosclerosis, osteoporosis, and skin atrophy) observed in patients with CRF.

The function and localization of another kidney-specific gene found in these cells, *SLC17A3*, have not been determined. However, NPT1, a sodium phosphate cotransporter, has 43.5% amino acid sequence identity with SLC17A3 and is the central molecule in the control of renal phosphate excretion (Shibui et al. 1999a, 1999b). Therefore, it is possible that SLC17A3 has a function similar to that of NPT1 and is expressed in kidney.

AQP2 (AQUAPORIN 2) was identified in the cultured cells and encodes a water channel in the apical membrane of the proximal renal tubule and collecting tubule in rats (Fushimi et al. 1993). Its expression in *Xenopus* oocytes markedly increases osmotic water permeability. The function and limited localization

TABLE 2

Expression of kidney-specific genes. Averages and standard deviations of the five selected kidney-specific genes are shown. Since the Affymetrix GeneChip analyzes expression based on the ratio between perfectly matched and mismatched probe readouts, there is no simple cutoff for the expression level. These five kidney-specific genes were calculated as positive

Gene	Expr.	Average	Stndrd Dev.
KL	Pos.	7.8	0.7
SLC17A3	Pos.	11.8	1.9
AQP2	Pos.	12.0	5.9
SLC22A2	Pos.	14.7	3.0
KCNJ1	Pos.	10.4	1.0

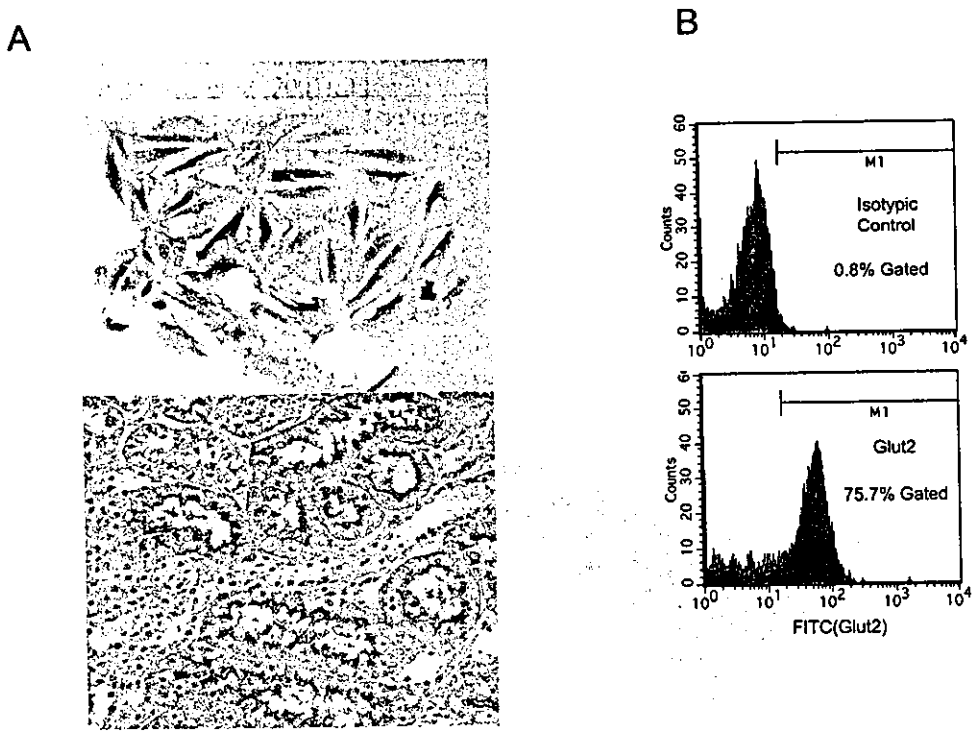

FIG. 1. The gamma-GTP and Glut2 staining of purified cells. In order to determine the origin of the purified cells, we examined two conventional renal tubular markers: gamma-GTP and Glut2. A: The primary cultured cells were positive for gamma-GTP enzymatic activity when stained as described in the Materials and Methods. The lower panel in the figure shows rat renal tubule tissue stained simultaneously to monitor the staining procedure. Since gamma-GTP is generally concentrated along the brush border, the interior of the duct structure shows high enzymatic activity. Conversely, cultured cells did not form a duct under our experimental conditions and moderate enzymatic activity was detected throughout the cells. B: Glut2 expression was analyzed using flow cytometry. As shown in the figure, 75.7% of the cells were positive for Glut2. The average and standard deviation in all cases were 62.6 and 13.3%, respectively.

of AQP2 suggest that it is a vasopressin-regulated water channel. The human homolog and the rat AQP2 share 91% amino acid identity (Sasaki et al. 1994). A defect in this gene is believed to be the basis of the autosomal dominant form of nephrogenic diabetes insipidus (Deen et al. 1994). AQP2 is detectable as both soluble and membrane-bound forms in the urine. In normal subjects, an infusion of desmopressin increases the urinary excretion of AQP2. The administration of vasopressin increased the urinary excretion by AQP2 in five patients with central diabetes insipidus, but not in four patients with X-linked or autosomal nephrogenic diabetes insipidus (Kanno et al. 1995).

SLC22A2 (Solute carrier family 22, member 2), also called *OCT2* (organic cation transporter2), was present in the cultured cells. Polyspecific organic cation transporters are critical for the elimination of many endogenous amines, exogenous drugs, and environmental toxins. Using PCR with primers derived from rat OCT1, human *OCT1* and *OCT2* were cloned from human kidney cortex cDNA (Gorboulev et al. 1997). While human *OCT2* expression was detected by RT-PCR in a few tissues, Northern blots revealed 2.5- and 4.0-kb OCT2 mRNAs mainly in the kidney (Gorboulev et al. 1997). In situ hybridization and immunohistochemistry revealed OCT2 localized at the luminal membrane of the kidney distal tubule (Gorboulev et al. 1997). *Xenopus* oocytes expressing *OCT2* showed increased cation uptake.

The fifth kidney-specific gene we found in the cultured cells was *KCNJ1* (an inwardly rectifying potassium channel,

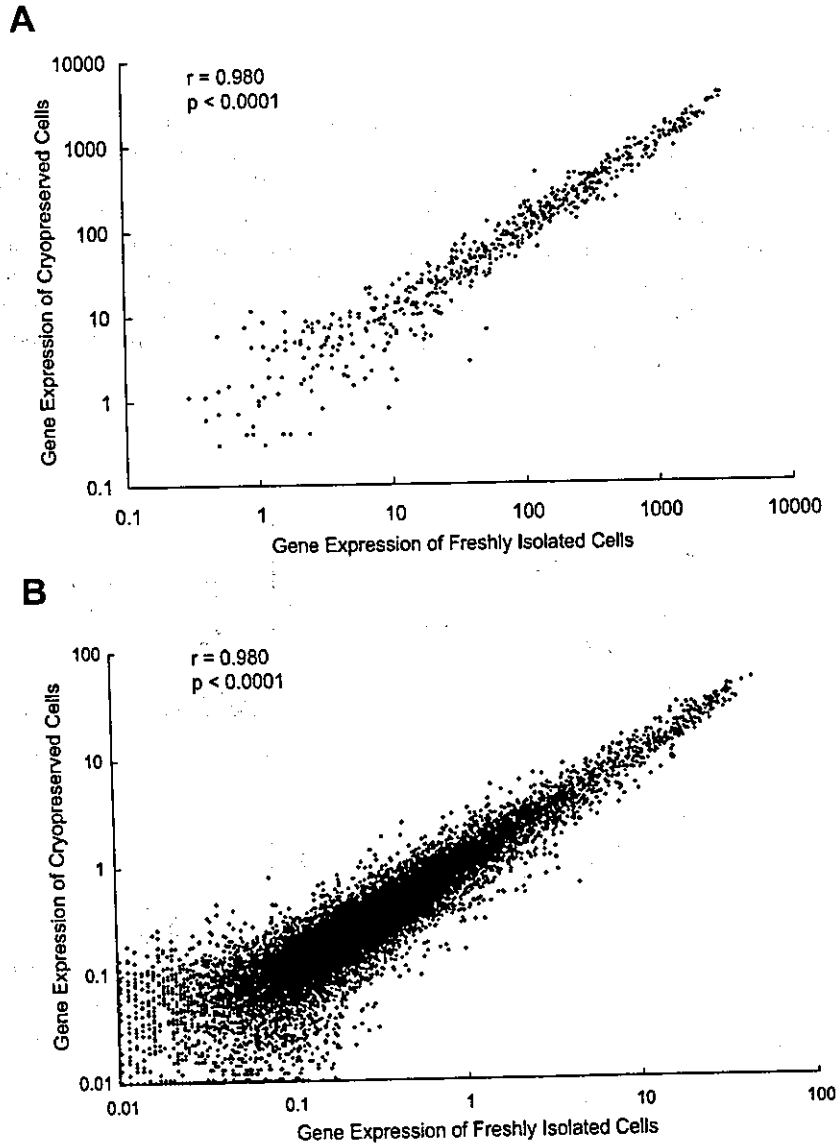

FIG. 2. The effects of cryopreservation. A: The gene expression data for 567 selected genes in cells with and without cryopreservation were compared without normalization ($r = 0.980$; $p < 0.0001$). B: Expression data for more than 39,000 genes were analyzed after normalization, as described in Materials and Methods. The removal of genes with low control signals as determined by the Cross Gene Error Model resulted in 16,147 genes; the expression data are shown here. There were no significant ($p < 0.05$) changes in the expression of any of the 16,147 genes according to nonparametric tests and the Benjamini and Hochberg False Discovery Rate for multiple testing.

subfamily J, member 1), also called *ROMK* (renal outer-medullary potassium channel), *ROMK1*, or *KIR1.1*. Inwardly rectifying potassium channels are important regulators of resting membrane potential and cell excitability. Using targeted mutations in *KCNJ1*, residues important for phosphatidylinositol 4,5-bisphosphate (PIP2) interaction were identified. Mutations in these residues associated with Andersen syndrome and Bartter's syndrome decreased channel-PIP2 interactions (Lopes et al. 2002).

Since the cells were purified from resected kidneys, the expression of kidney-specific genes is not surprising. However, these results indicate that the cells retained the characteristics of renal components after a few weeks in culture and the major population of cells in the cultures was not leukocytes or other passengers that happened to be harvested and concentrated by the artificial purification conditions.

In addition to mRNA expression, we tested two cell type-specific markers: gamma-GTP enzymatic activity and Glut2 antigen expression. At least two commercial suppliers of human proximal renal tubular cells (Cambrex Corporation, Rockland, ME, and TaKaRa Bio Inc., Ohtsu, Japan) use gamma-GTP staining for quality control. Therefore, gamma-GTP activity is regarded as a standard marker of human proximal renal tubular cells among renal tissues; it is also known as a bile duct marker. As shown in Figure 1a, the primary cultured cells were positive for gamma-GTP. Conversely, Glut2 was originally reported to be expressed in human liver, pancreatic islet cells, and the kidney (Fukumoto et al. 1988); its expression is considered to reflect renal tubular origin in kidney (Vestri et al. 2001). Flow cytometric analysis indicated that the major population of cultured cells (75.7%) expressed Glut2 on their cell surface membranes (Fig. 1b). The average and standard deviation of Glut2 expression in all cases were 62.6 and 13.3%, respectively. Based on these findings, the majority of the purified and cultured cells originated from the proximal renal tubule and retained some of the characteristics of the original tissue.

Effects of Cryopreservation

Cells in an initial primary culture can grow to cover the culture dish surface and then be removed and plated at a lower density to form a secondary culture. This process can be repeated several times, but after five to six weeks in culture, the cell morphology changes, and the viability is reduced. Therefore, primary cultures demand timely experiments. However, given the limited number of surgical operations and the limited space and time for experiments, studies with cultured cells would be more feasible if cryopreserved cells could be used. To assess the effect of cryopreservation, we compared freshly prepared primary cultures of renal tubular cells with cells that had been cryopreserved, thawed, and then cultured. First, we compared the expression of housekeeping genes before and after cryopreservation and analyzed the readout from the scanner without normalization. Pearson's correlation coefficient (r) for the 567 housekeeping genes was 0.980, and the p-value was less than 0.0001 (Fig 2). Housekeeping genes are defined as those constitutively expressed to maintain cellular functions and are presumed to be expressed at the same levels as long as cells maintain their essential functions. A comparison of all 39,000 scanned transcripts before and after cryopreservation by nonparametric statistical analysis after normalization showed that no gene expression was changed significantly ($p < 0.05$). Due to the nature of the detection system, there was some variability, especially in weakly expressed genes; this variation was determined to be insignificant using the Cross Gene Error Model. Therefore, essential cell functions appear unchanged by cryopreservation; we will use cryopreserved cells for future experiments.

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We also examined RTKN2 expression in haemopoietic malignancies. It is low or absent in myeloid leukaemias and low in most B cell malignancies, with the exception of high expression of Variant-3 in a case of mantle cell lymphoma. It is also low in CD8+ T-cell malignancies, but extremely high in two cases of CD4+CD8+ T-lymphoblastic lymphoma and in Jurkat cells.

Rho/Rac GTPases are critical in lymphocyte development and function, though relatively little is known about the function and role of effector proteins. We hypothesize that RTKN2 may play an important role in mediating the effects of the Rho GTPases in specific subsets of lymphoid cells, and perhaps in some lymphoproliferative disorders. As RTKN2 was first identified in a cell line resistant to apoptosis, its role in modulating apoptotic signals in lymphoid cells may be of particular importance.

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EXPRESSION PROFILING OF GENE WITH UPSTREAM AML1 RECOGNITION SEQUENCE IN HEMATOPOIETIC STEM CELL-LIKE FRACTIONS FROM INDIVIDUALS WITH THE M2 SUBTYPE OF HUMAN ACUTE MYELOID LEUKEMIA

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AML1 gene is one of the most frequent targets for chromosomal translocations associated with acute myeloid leukemia. While AML1 is a transcription factor, which is critical for definitive hematopoiesis and leukemogenesis, little has been known about genes under the control of AML1 or the fusion proteins, and mechanism of their regulation.

In this study, we searched whole genome sequence and obtained a table of upstream sequences related to the gene identifiers. Thereafter, we scanned the table to generate a list of genes which have an AML1 recognition sequence in their upstream. We analyzed expression profiles of the listed genes of hematopoietic stem cell-like fractions from individuals with the M2 subtype of human acute myeloid leukemia with (n=5) and without (n=15) the distinct chromosomal translocation, t(8;21). We examined genes whose expression level differed significantly between two groups (Welch ANOVA test, $P<0.01$). A total of 276 genes were identified (151 and 125 genes were upregulated and down-regulated in the t(8;21) positive group, respectively). In this study, most of known genes, which have AML1 recognition sequence at their upstream, including M-CSF receptor, IL-3, T-cell receptor alpha chain, immunoglobulin mu heavy chain, defensin NP-3, neutrophil elastase and myeloperoxidase, were upregulated in t(8;21) positive group, and rest of them including T-cell receptor beta chain and GM-CSF produced controversial results between probe sets within each transcript. These results suggest that most genes previously reported as a target for AML1 transcription factor are upregulated by the existence of the translocation, t(8;21). There are some other well known translocations or fusion genes including t(15;17) translocation, bcr-abl fusion gene, translocation at 11q23, etc. Our strategy to analyze a relationship between these expression data and genes with upstream transcription factor recognition sequences may be useful to compile new knowledge in these fields.

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IDENTIFICATION OF NEIGHBORHOODS OF GENES EXPRESSED BY HEMATOPOIETIC STEM CELLS

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The gene expression pattern of hematopoietic stem cells (HSC) was examined in baboons. In order to deplete committed hematopoietic progenitors animals received sublethal total body irradiation (250 cGy). All baboons (n=4) became severely leukopenic and thrombocytopenic from day 7-21, however, blood counts returned to normal by day 35-49 indicating the presence of radioresistant HSCs. Marrow CD34+ cells were reduced by 75% from day 7-35 but approached normal numbers by day 85. Colony forming cells (CFC) and cobblestone area-forming cells (CAFC) remained reduced by 95% until day 49. The day 28 CD34+ cells were used for transcriptional profiling using Affymetrix oligonucleotide arrays and an activity center algorithm which permits identification of functionally related families of genes. These CD34+ cells represent a primitive subpopulation of HSC since they were largely incapable of forming hematopoietic colonies or cobblestone areas yet they were responsible for hematological reconstitution. There was downregulation of genes involved with mRNA splicing, protein folding (chaperones), ribosomal function and translational initiation (day 28) which recovered by day 49. Seven families of genes that were overexpressed to a statistically significant degree in day 28 CD34+ cells were: (1) secreted factors and receptors such as IL-11, bone morphogenetic proteins (BMP), BMP receptors, smoothened, ciliary neurotrophic factor (CNTF), stromal derived factor-1 (SDF-1) (2) the complement system proteins (C1qRp); (3) cell adhesion molecules (CD44, ICAM, matrix metalloproteinases); (4) anti-apoptotic genes (bcl-2); (5) DNA damage and repair genes; (6) PIP2 activated K+ channels; (7) adenylate cyclases. In addition, these CD34+ cells expressed genes that are characteristic of a broad variety of non-hematopoietic tissues such as liver (C1qRp, albumin, fibrinogen), brain (CNTF, GABA/Glycine receptor) and marrow stroma (SDF-1, fibronectin, collagen etc). These studies provide insight into the genetic profile of radioresistant HSC and their ability to express genes characteristic of several non-hematopoietic tissues.

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PERCENT CO-EXPRESSION OF CD52 AND CD20 ANTIGENS AND MEAN FLUORESCENCE INTENSITIES (MFI) ARE IMPORTANT FACTORS IN THE ADDITIVE EFFECT OF ANTIBODY-INDUCED APOPTOSIS IN CHRONIC LYMPHOCYTIC (CLL) AND NON-HODGKIN'S LYMPHOMA (NHL) CELLS: IMPLICATIONS TO ANTIBODY-BASED THERAPY

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CD20 (Rituximab) and CD52 (Campath1-H) antibodies (AB) have been employed in the treatment of NHL and CLL patients (pts), as single agents or in combination with chemotherapy. Rituximab has been effective in NHL pts, whereas Campath 1-H has been effective in CLL pts, however, only limited response was observed for Campath 1-H. We hypothesized that responsiveness to a single or combined AB therapy depends on the extent of expression of the relevant AB for each disease. We therefore determined the extent of apoptosis by CD20, CD52 and CD52+CD20 AB of blood mononuclear cells (MNC) from 10 healthy donors (HD); 17 CLL patients; and 35 bone marrow MNC from NHL pts. Apoptosis (by Annexin V) was correlated to % expression of CD20, CD52, or CD20/CD52 and to the MFI.

CD52 was highly expressed in all pts' samples. However, significant differences were observed in MFI between the 3 groups. MFI were 449 ±48 fluorescence units (FU); 70±22 and 158±81 FU, in HD, NHL and CLL pts, respectively. Mean %CD20 expression was 32±4%; 51.4±15% and 28±8%, respectively and MFI was 62±14 FU;19±8 and 35±14 FU, respectively. Mean co-expression of CD20+CD52 was 26±3%; 31±13%; and 48±21%, respectively.

Mean apoptosis by CD52, CD20 and CD52+CD20 AB for HD was 25,000+18,000; 23,000±21,000 and 47,000±23,000 apoptotic cells/ug antibody/24h treatment, respectively. For CLL was 17,000±16,000; 20,000±20,000 and 25,000±22,000, respectively. For NHL was 11,000±7,200; 16,000±7,000 and 13,000±19,000 cells, respectively.

Significant correlation was observed between MFI and apoptosis for CD 52 ($R=0.5$; $p=0.02$) in CLL pts and for CD20 in NHL pts ($R=0.6$; $p=0.004$). The combination of CD20+CD52 significantly increased apoptosis for CLL and NHL pts.

Conclusions: 1. Pretreatment screening for CD52 and CD20 is required for CLL and NHL pts. 2. The combination of CD52 and CD20 is effective in pts with high expression of both antigens.

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RECOMBINANT RETROVIRUSES AS TOOLS FOR IDENTIFYING NEW ONCOGENES ASSOCIATED WITH LYMPHOMA

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The recombinant retrovirus, MoFe2-MuLV, was constructed by replacing the U3 region of Moloney murine leukemia virus (MoMuLV) with homologous sequences from the feline leukemia virus (FeLV-945) LTR. Like other gammaretroviruses, MoMuLV and FeLV induce lymphoma in the natural host through insertional activation of host