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Proteomic and Transcriptomic Analyses of Retinal Pigment Epithelial Cells Exposed to REF-1/TFPI-2

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Purpose. The authors previously reported a growth-promoting factor, REF-1/TFPI-2, that is specific to retinal pigment epithelial (RPE) cells. The purpose of this study was to determine the genes and proteins of human RPE cells that are altered by exposure to TFPI-2.

Methods. Human primary RPE cells were cultured with or without TFPI-2. Cell extracts and isolated RNA were subjected to proteomic and transcriptomic analyses, respectively. Proteins were separated by two-dimensional gel electrophoresis followed by gel staining and ion spray tandem mass spectrometry analyses. Transcriptomic analysis was performed using a DNA microarray to detect 27,868 gene expressions.

Results. Proteomic analysis revealed c-Myc binding proteins and ribosomal proteins L11 preferentially induced by TFPI-2 in human RPE cells. Transcriptomic analysis detected 10,773 of 33,096 probes in the TFPI-2 treated samples, whereas only 2186 probes were detected in the nontreated samples. Among the genes up-regulated by TFPI-2 at the protein level were *c-myc*, Mdm2, transcription factor E2F3, retinoblastoma binding protein, and the *p21* gene, which is associated with the *c-myc* binding protein and ribosomal protein L11.

Conclusions. The mechanisms by which TFPI-2 promotes the proliferation of RPE cells may be associated with augmented *c-myc* synthesis and the activation of E2F in the retinoblastoma protein (Rb)/E2F pathway at the G1 phase of the RPE cells. Activation of ribosomal protein L11 and the Mdm2 complex of the p53 pathway may be counterbalanced by the hyperproliferative conditions. (*Invest Ophthalmol Vis Sci.* 2007;48: 516–521) DOI:10.1167/iovs.06-0434

Retinal pigment epithelial (RPE) cells play important roles in maintaining the homeostasis of the retina. RPE cells, located between the sensory retina and the choroidal blood supply, form a diffusion barrier controlling access to the subretinal space, with the RPE membrane regulating the transport of proteins and controlling the hydration and ionic composition of the subretinal space. The sensitivity and viability of the photoreceptors thus depend on RPE-catalyzed transport activity. Proteins in the RPE cells that function in ionic, sugar, peptide, and water transport have been identified.[1] Damage to RPE cells generally leads to degeneration of the neural retina, as occurs in retinitis pigmentosa and age-related macular degeneration. Transplantation of the healthy retinal pigment cells or embryonic stem cells differentiating into RPE cells would be an ideal therapeutic approach to treat such diseases, and such attempts have been made.[2]

An alternative approach to treat these retinal diseases would be the use of a growth factor that promotes proliferation of the remaining RPE cells in a damaged retina or one that stimulates the regeneration of damaged RPE cells. To find such factor(s), the proteins expressed in human fibroblast cells were fractionated and assayed, leading to the isolation of RPE cell factor-1 (REF-1), which selectively promoted the proliferation of primary human RPE cells.[3]

Subsequently, the cDNA of REF-1 was cloned using information from the N-terminal amino acid sequences, which was identical with the tissue factor pathway inhibitor-2 (TFPI-2).[3] Earlier studies have shown that TFPI-2 is a Kunitz-type serine protease inhibitor[4–6] involved in the regulation of extrinsic blood coagulation[4,7] and in the proliferation, invasion, and metastasis of various types of malignant cells.[4,8–13] Extensive studies on the physiological roles of TFPI-2 have revealed that the ERK/MAPK pathway[13] may be associated with the up-regulation of the *TFPI-2* gene and that DNA methylation[9,10] in certain tumor cell lines may be related to the downregulation of the *TFPI-2* gene. When TFPI-2 is added to the culture medium of vascular smooth muscle cells, it promotes cell proliferation.[14]

Our initial finding that TFPI-2 enhanced RPE proliferation prompted us to question how this was achieved. We applied proteomic and transcriptomic analyses to screen the changes in the expression of the RNAs and proteins in RPE cells and will show that the proliferation promoting activity of TFPI-2 on RPE cells is associated with the regulation of an oncogene product, *c-myc*, and representative cancer repressor proteins retinoblastoma protein (Rb)/E2F and p53.

Materials and Methods

TFPI-2 Treatment of Human RPE Cell Culture

Human primary RPE cells (passage 5) were seeded at a density of 2.5×10^4 cells/0.5 mL per well in 24-well plastic plates (BD Biosciences, Franklin Lakes, NJ) with Dulbecco modified MEM (DMEM; Invitrogen Japan, Tokyo, Japan) containing 15% fetal calf serum (FCS, Invitrogen). TFPI-2 was added to 20 wells with the RPE cells at 10 ng/mL concentrations and was incubated at 37°C for 24 hours for the proteomic samples, and for 6 hours, 12 hours, and 24 hours for the transcriptomic samples. An equal amount of saline was added to 20 wells containing RPE cells for controls. TFPI-2 was donated by Toray Industries, Inc., Tokyo, Japan.

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Protein Sample Preparation

To isolate whole cellular protein extracts from cultured RPE cells, the cells were rinsed 3 times with 1× PBS (pH 7.4) and were lysed in a denaturing lysis buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 40 mM Tris, 0.2% purifier (Bio-Lyte, pH range 3–10; Bio-Rad, Hercules, CA), and 50 mM dithiothreitol (DTT). The collected lysate was then centrifuged at 14,000*g* for 15 minutes at 4°C. Proteins in the supernatant were repeatedly concentrated and precipitated and finally desalinated (Readyprep 2-D Cleanup kit; Bio-Rad). The protein concentration in the RPE samples was determined by a modified Lowry method adapted for use with the lysis buffer.

Two-Dimensional Electrophoresis

Protein samples were separated by a two-dimensional electrophoresis method. A 300-μg protein sample was loaded on immobilized pH gradient (IPG) strips (pH 3–10, 7 cm; pH 4–7, 17 cm; Bio-Rad) by in-gel rehydration at 20°C overnight. For the 7-cm strip, isoelectric focusing (IEF) was used for the first dimension at an initial voltage of 250 V for 15 minutes, increased to 4000 V for 2 hours, and held until 20,000 V/h was reached. For the 17-cm strip, the initial voltage was set at 250 V, as for the 7-cm strip. Then the voltage was increased to 10,000 V for 3 hours and was held until 60,000 V/h was reached. Immediately after IEF, the IPG strips were equilibrated for 20 minutes in buffer containing 6 M urea, 2% SDS, 0.375 M Tris (pH 8.8), and 20% glycerol under a reduced condition with 2% DTT (Bio-Rad), followed by another incubation for 10 minutes in the same buffer under alkylating conditions with 2.5% iodoacetamide (Bio-Rad).[15]

Equilibrated IPG strips were then electophoresed by SDS-PAGE for the second dimension. Images of the chemiluminescent signals were captured and merged with those of protein spots made visible by protein gel stain (Sypro Ruby; Bio-Rad), and the spots corresponding to the immunoreactivity were cut out. To test reproducibility, the experiment was performed twice.

Protein Identification by Mass Spectrometry

Excised gel pieces were rinsed with water and then with acetonitrile and were completely dried for the reduction-alkylation step. They were incubated with 10 mM DTT in 100 mM ammonium bicarbonate for 45 minutes at 56°C, then with 55 mM iodoacetamide in 100 mM ammonium bicarbonate for 30 minutes at room temperature in the dark. The supernatant was removed, and the washing procedure was repeated three times. Finally, the gel pieces were again completely dried before trypsin digestion and were rehydrated in a solution of trypsin (12.5 ng/μL; Promega, Madison, WI) in 50 mM ammonium bicarbonate. The digestion was continued for 16 hours at 37°C, and the extraction step was performed once with 25 mM ammonium bicarbonate, then twice with 5% formic acid, and finally with water. After resuspension in 40 μL solution of aqueous 0.1% trifluoroacetic acid/2% acetonitrile, the samples were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). For analysis by LC-MS/MS, the tryptic digests were injected by an automatic sampler (HTS-PAL, CTC Analytics, Zwingen, Switzerland) onto a 0.2 × 50-mm capillary reversed-phase column (Magic C18, 3 μm; Michrom BioResources, Inc., Auburn, CA) using an HPLC (Paradigm MS4; Michrom BioResources). Peptides were eluted with a gradient (95% solvent A consisting of 98% H_2O/2% acetonitrile/0.1% formic acid)/5% solvent B (10% H_2O/90% acetonitrile/0.1% formic acid; 0 minute)/35% solvent A/65% solvent B (20 minutes)/5% solvent A/95% solvent B (21 minutes)/5% solvent A/95% solvent B (23 minutes)/95% solvent A/5% solvent B (30 minutes) for 30 minutes at a flow rate of 1.5 μL/min. Peptides were eluted directly into an ion trap mass spectrometer (ESI; Finnigan LTQ; Thermo Electron Corporation, Waltham, MA) capable of data-dependent acquisition. Each full MS scan was followed by an MS/MS scan of the most intense peak in the full MS spectrum with the dynamic exclusion enabled to allow detection of less-abundant peptide ions. Mass spectrometric scan events and HPLC solvent gradients were controlled with the use of a computer program (Paradigm Home; Michrom BioResources).

Total RNA Isolation from RPE Cells

Total RNA was isolated from the cultured RPE cells after 6 hours, 12 hours, and 24 hours with TFPI-2 using a total RNA isolation kit (RNA-Bee-RNA Isolation Reagent; Tel-Test, Friendswood, TX). Total RNA samples were treated with RNase-free DNase (Roche Diagnostics Japan) to minimize genomic DNA contamination.

DNA Microarray Analysis

DNA microarray analysis was performed (AB1700 Chemiluminescent Microarray Analyzer; Applied Biosystems, Foster City, CA). The survey array used (Human Genome Survey Array; Applied Biosystems) contained 33,096 60-mer oligonucleotide probes representing a set of 27,868 individual human genes and more than 1000 control probes. Sequences used for the microarray probe were obtained from curated transcripts (Celera Genomics Human Genome Database), RefSeq transcripts that had been structurally curated from the LocusLink public database, high-quality cDNA sequences from the Mammalian Gene Collection (MGC; http://mgc.ncl.nih.gov), and transcripts that were experimentally validated (Applied Biosystems). The 60-mer oligo probes were synthesized using standard phosphoramidite chemistry and solid-phase synthesis and underwent quality control by mass spectrometry. The probes were deposited and covalently bound to a derivatized nylon substrate (2.5 × 3 inches) that was backed by a glass slide by contact spotting with a feature diameter of 180 μm and more than 45 μm between each feature. A 24-mer oligo internal control probe (ICP) was cospotted at every feature with 60-mer gene expression probe on the microarray. Digoxigenin-UTP labeled cRNA was generated and linearly amplified from 1 μg total RNA (Chemiluminescent RT-IVT Labeling Kit, version 2.0; Applied Biosystems) according to the manufacturer's protocol. Array hybridization (two arrays per sample), chemiluminescence detection, image acquisition, and analysis were performed (Chemiluminescence Detection Kit and AB1700 Chemiluminescent Microarray Analyzer; Applied Biosystems) according to the manufacturer's protocol.

Briefly, each microarray was first prehybridized at 55°C for 1 hour in hybridization buffer with blocking reagent. Sixteen micrograms labeled cRNA targets were first fragmented into 100 to 400 bases by incubation with fragmentation buffer at 60°C for 30 minutes, mixed with internal control target (ICT; 24-mer oligo labeled with LIZR fluorescent dye), and hybridized to each prehybrid microarray in 1.5 mL vol at 55°C for 16 hours. After hybridization, the arrays were washed with hybridization wash buffer and chemiluminescence rinse buffer. Enhanced chemiluminescent signals were generated by first incubating the arrays with anti-digoxigenin alkaline phosphatase and enhanced with chemiluminescence enhancing solution and chemiluminescence substrate.

Images were collected from each microarray using the 1700 analyzer equipped with a high-resolution, large-format CCD camera, including 2 "short" chemiluminescent images (5-second exposure length each) and 2 "long" chemiluminescent images (25-second exposure length each) for gene expression analysis, two fluorescent images for feature finding and spot normalization, and two quality control images for spectrum cross-talk correction. Images were quantified, corrected for background and spot, and spatially normalized.

Data Analysis

MS data were identified with the use of a protein search program (BioWorks 3.2; Thermo Electron Corporation, Waltham, MA). For protein database searches, the same program was used to create centroid peak lists from the raw spectra. These peak lists were then submitted for database searching (BioWorks). The identity of the samples was searched from databases (nrNCBI [www.ncbi.nlm.nih.gov]) that extracted proteins and were restructured; search terms included human and *Homo sapiens*. Differentially expressed proteins were further analyzed for related genes and proteins using natural language processing software (Pubgene database; PubGene Inc., Boston, MA) and data mining software of gene expression (OmniViz; OmniViz, Inc., Maynard, MA).

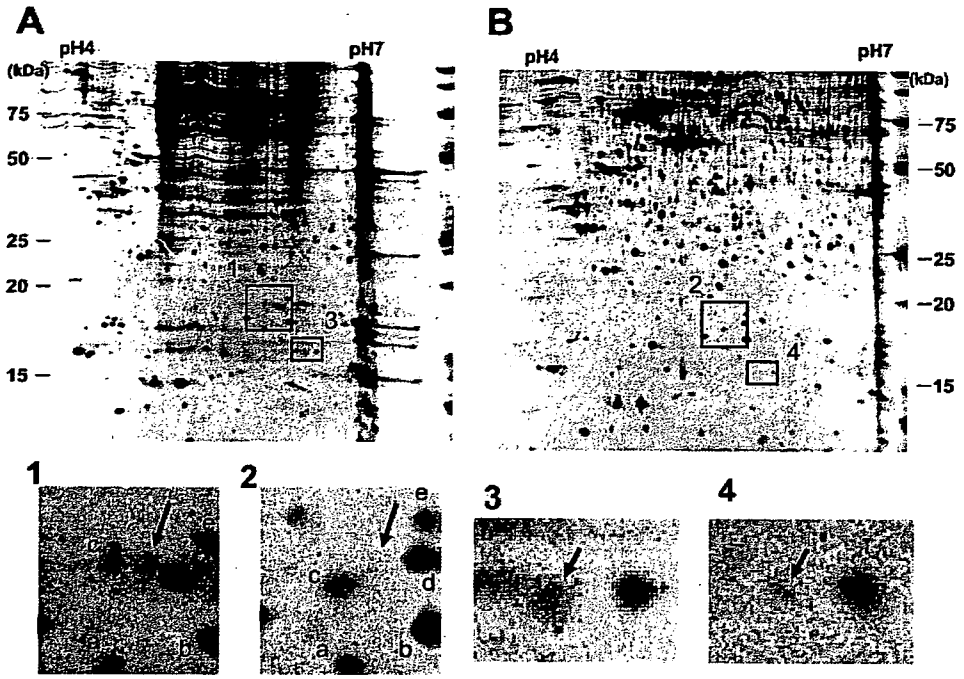

FIGURE 1. Two-dimensional gel electrophoresis of human RPE cells culture with (A) and without (B) TFPI-2. Spots corresponding to proteins whose expression is dependent on the presence of TFPI-2 in the culture medium are indicated by the *arrows* (*insets*). Proteins were detected by SYPRO Ruby staining. Spots corresponding to the differentially expressed proteins indicated by *arrows* (1 vs. 2 and 3 vs. 4) were subsequently subject to the LC-MS/MS analysis so that proteins could be identified.

RESULTS

Proteome Analysis of RPE Cells Treated with TFPI-2

To determine the mechanisms responsible for the proliferation-promoting activity of TFPI-2 on RPE cells, protein synthesis and RNA expression were determined before and after TFPI-2 exposure. Differentially expressed proteins in the primary human RPE cells in response to TFPI-2 were identified by two-dimensional electrophoresis (Fig. 1). Samples were initially separated using IPG at a pH range of 3 to 10 to observe the full distribution of protein spots. The pH range was then narrowed to 4 to 7 to obtain higher resolution for spot picking. Consequently, approximately 480 spots were identified in the whole gel. We then focused on molecular weight less than 25 kDa, which is easy to check for changes. Ten spots considered differentially expressed in the two-dimensional gel were collected and subjected to LC-MS/MS analysis. Among the identified proteins, ribosomal protein L11 (RPL11; Fig. 1-1) and c-Myc binding protein (MYCBP; Fig. 1-3), known for regulating cell proliferation, were identified.[16] These two proteins, identified by LC-MS/MS analysis and data analysis software (Bioworks 3.2), were consistent with those estimated from the results of two-dimensional electrophoresis (Table 1).

Transcriptomic Analysis of RPE Cells Treated with TFPI-2

The expression of 8134 genes in RPE cells was analyzed using DNA microarray with and without TFPI-2 exposure for 6 hours, 12 hours, and 24 hours. Signal normalization was performed for six independent DNA microarray chips according to the manufacturer's protocol. Genes differentially expressed by more than threefold were considered significant and were selected for further analysis. Among the 33,096 possible probes, 10,773 probes were detected in the RPE cells incubated with TFPI-2, whereas only 2186 probes were detected without TFPI-2. Based on expression levels at the three time points (6 hours, 12 hours, and 24 hours), the time-dependent expression pattern of each gene was calculated and clustered with other genes with similar expression patterns using data mining software (OmniViz). Data analysis resulted in 38 clusters of genes that either increased or decreased their expression levels by more than twofold after TFPI-2 (Fig. 2). Nineteen genes were upregulated in 5 clusters, 108 genes in 16 clusters, and 717 genes in 22 clusters at 6 hours, 12 hours, and 24 hours, respectively. For downregulated genes, 30 genes in 16 clusters, 119 genes in 19 clusters, and 3 genes in 19 clusters were observed after 6 hours, 12 hours, and 24 hours, respectively. Transcriptomic analysis revealed significantly more genes differentially expressed at the transcriptional level than at the proteome level.

DISCUSSION

Proteins and genes whose expression was upregulated or downregulated after exposure to TFPI-2 were analyzed in human RPE cells to study the proteomic and transcriptomic changes. Protein and gene expression profiles for human RPE cells have been reported by West et al.,[17] who identified 278 proteins, and Cai et al.,[18] who reported 5580 ± 84 genes expressed in adult human RPE and ARPE19 cell lines using a DNA chip with 12,600 probes (Human U95Av2; Affymetrix, Santa Clara, CA). Our study showed changes in the expression of 8134 of 27,868 genes. DNA microarray analyses were simul-

TABLE 1. Two-Dimensional Gel Spots Identified by Mass Spectrometry

Protein	Number of AA	Peptide Residues	Identified Peptide from Database	MW	Score	Accession Number
c-Myc binding protein	167	108–117	TAEDAKDFFK	18642.6	10.13	1731809
Ribosomal protein L11	177	88–94	VREYELR	20125.1	20.21	14719845

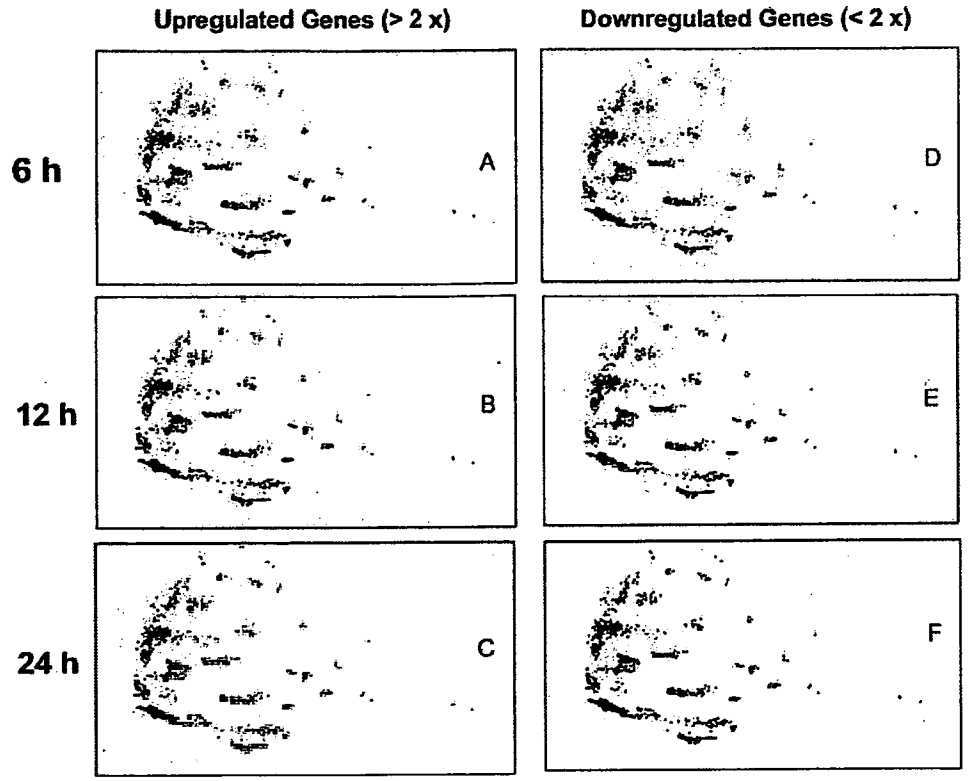

FIGURE 2. Differentially expressed genes detected by DNA array are plotted as clusters. Differentially expressed genes whose expression level was increased by more than twofold (A–C) or was reduced by more than 0.5-fold (D–F) in RPE cells treated with TFPI-2 at incubation times of 6 hours, 12 hours, and 24 hours compared with the control cells are shown. Expression profile analysis revealed different gene expression patterns at each incubation time.

taneously performed at three time points (6 hours, 12 hours, and 24 hours) to monitor the course of expression of the possible 27,868 genes in human RPE cells exposed and not exposed to TFPI-2. This study was conducted at the translational and the transcriptional levels to complement the disadvantages of each method.

Raw gene expression data were further analyzed with data mining software (OmniViz) to obtain an overall picture of the transcriptional changes induced by TFPI-2 in human primary RPE cells. Genes whose expressions were changed by more than twofold were clustered into 38 groups showing a change of expression at each time point (Fig. 2). The number of genes upregulated at each time point was considerably higher than the number that was downregulated. A small number of genes was triggered by TFPI-2 treatment at 6 hours, before the major changes occurred at 24 hours. Among the initially upregulated genes were reticulon 4 interacting protein 1, phospholipase C, delta 1, granzyme M (lymphocyte met-ase 1; *GZMM*), and mitochondrial ribosomal protein L41 (*MRPL41*).

Proteomics analysis simultaneously performed at 24 hours identified two differentially expressed proteins, the *c-myc* binding protein (MYCBP) and the ribosomal protein L11 (RPL11). MYCBP and RPL11 (Fig. 3) are well known to regulate cell cycling through the Rb/E2F pathway and the p53 pathway, respectively. MYCBP stimulates *c-myc* transcription through the retinoblastoma protein (Rb)/E2F pathway (see Fig. 5). Sears et al.[19] reported that activation of Myc increased the signal transduction of the cyclin D/cdk4 and cyclin E/cdk2 pathways. Activation of these pathways inactivates Rb after phosphorylation and E2F dissociation, which then promotes RPE cells to go into the S-phase of the cell cycle. The twofold transcriptional increase of *Rb* and *E2F3* in TFPI-2 exposed cells compared with control at 24 hours supports this hypothesis (Figs. 4C, 4F).

Concomitantly, the expressions of Rb and Mdm2 were upregulated twofold in growth-stimulated cells compared with control cells. Because Rb is associated with the negative regulation of the G_1-phase of the cell cycle, the enhanced expres-

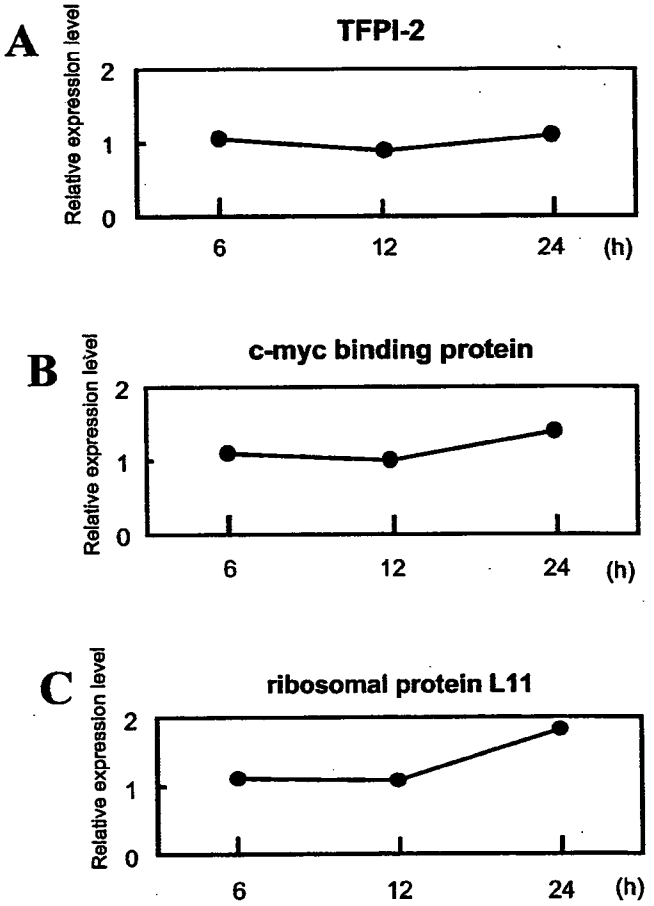

FIGURE 3. Time course of gene expression for TFPI-2 (A), *c-myc* binding protein (B), and ribosomal protein L11 (C) in the cultured human RPE cells after exposure to TFPI-2.

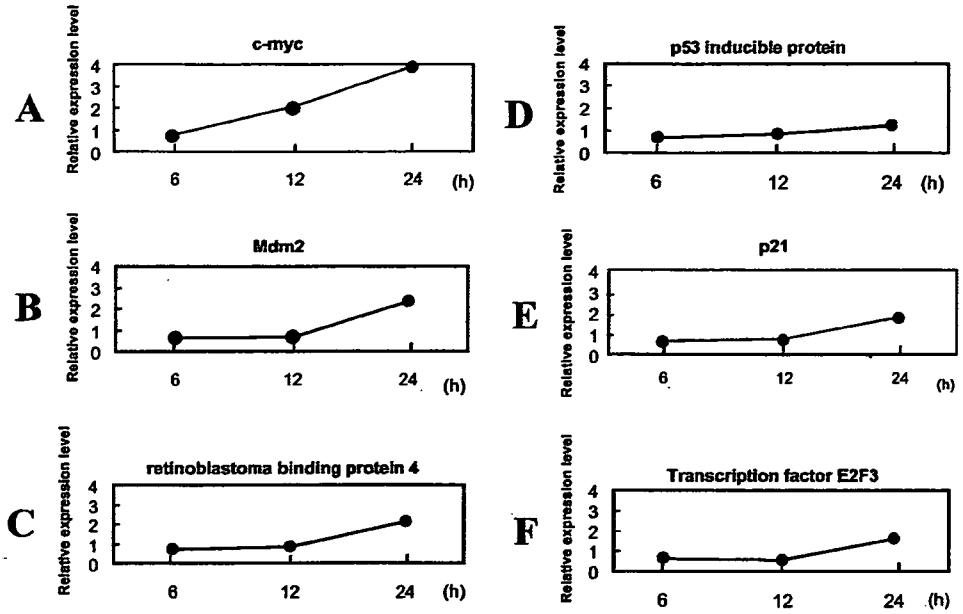

FIGURE 4. Time courses of protein expression patterns for *c-myc* (A), Mdm2 (B), retinoblastoma binding protein 4 (C), p-53 inducible protein (D), p21 (E), and transcription factor E2F3 in the cultured human RPE cells after exposure to TFPI-2.

sion of *Mdm2* might have been involved in the augmented degradation of Rb through the ubiquitin/proteasome-dependent pathway. Recently, Uchida et al.[20] suggested that Mdm2 regulates the function of RB through the ubiquitin-dependent degradation of RB.

The *Rb* gene was the first identified tumor-suppressor gene,[21] and it was recognized as a central component of a signaling pathway that controlled cell proliferation. Specifically, the D-type G_1 cyclins, together with their associated cyclin-dependent kinases (CKDs) Cdk4 and Cdk6, initiated the phosphorylation of Rb and Rb family members, inactivating their capacity to interact with the E2F transcription factors (Fig. 5).[19] This phosphorylation leads to an accumulation of E2F1, E2F2, and E2F3a, which activate the transcription of a large number of genes essential for DNA replication and further cell cycle progression.[22–26] Among the E2F targets are genes encoding a second class of G_1 cyclins, cyclin E, and the associated kinase Cdk2 (Fig. 5).[19] The activation of cyclin E/Cdk2 kinase activity by E2F leads to further phosphorylation and inactivation of Rb, further enhancing E2F activity and increasing the accumulation of cyclin E/Cdk2 (Fig. 5).[19] This feedback loop, which leads to a continual inactivation of *Rb* independent of the action of cyclin D/Cdk4—defined as a junction in cell proliferation response when passaged through the cell cycle—becomes growth factor independent.[25,26] The activity of the G_1 Cdks is negatively regulated by a family of cyclin-dependent kinase inhibitors (CKIs), including $p21^{WAF1}$, $p27^{Kip1}$, and the $p16^{INK4a}$ family.[27] The three upregulated E2Fs associate exclusively with Rb and appear to play a positive role in cell cycle progression.[19]

RPL11 binds the mouse double-minute 2 (Mdm2 is the mouse homologue of Hdm2 in humans) protein with other ribosomal proteins (L23 and L5) to form a complex to inhibit ubiquitin-dependent degradation of p53.[28–30] The RPL11 protein is expressed in ARPE-19 cells.[31] Inhibition of p53 degradation leads to p21 signaling, which participates in the G_1

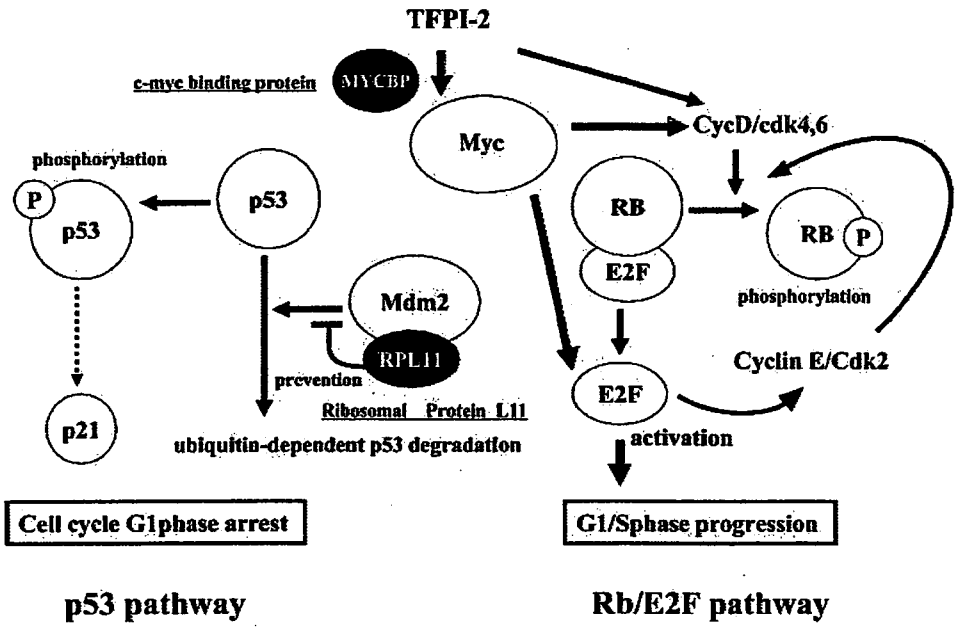

FIGURE 5. Hypothetical network of various genes and proteins associated with the growth-promoting effect of TFPI-2 on the human RPE cells. *Arrows*: stimulatory signals. *Straight* and *dotted lines*: inhibitory effects.

arrest of the cell cycle but also negatively regulates cell proliferation (Fig. 5).[30,32–34] In support of this hypothesis, *p21* transcription was increased by twofold after 24 hours by TFPI-2.

The *p53* gene mediates a major tumor-suppression pathway in mammalian cells and is frequently altered in human tumors.[30] Its function is kept at a low level during normal cell growth and is activated in response to various cellular stresses by acting as a sequence-specific transcription factor.[30] The p53 protein induces cell cycle arrest or apoptosis.[30]

Shinoda et al.[14] reported cell growth proliferation of vascular smooth muscle endothelial cells by a purified mitogenic substance from human umbilical vein endothelial cells, later identified as TFPI-2. These authors showed the rapid activation of mitogen-activated protein kinase (MAPK) by TFPI-2 and the induced activation of proto-oncogene *c-fos* mRNA in smooth muscle cells.[14] They concluded that *c-fos* activation was initiated by MAPK based on MAPK inhibitor PD098059 suppression.

In conclusion, the results of proteomic and transcriptomic analyses suggest that the proliferation of RPE cells induced by TFPI-2 is regulated through the Rb/E2F, p53, and Ras/Raf/MAPK pathways. We and others[3,35] have reported a transcript of TFPI-2 in the mRNA of RPE cells. It is now reasonable to expect that RPE cells are able to self-proliferate by generating TFPI-2. Additional studies are needed to determine whether TFPI-2 can act as such an autocrine factor and can be modified for future treatment of the dry-type age-related macular degeneration and of retinitis pigmentosa.

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Gastroenterology

Meta-Analysis of Risk of Malignancy with Immunosuppressive Drugs in Inflammatory Bowel Disease

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BACKGROUND: There is a concern as to whether long-term administration of immunosuppressants in patients with inflammatory bowel disease (IBD) would increase the risk of malignancy.

OBJECTIVE: To compare the risks of developing malignancy between patients with IBD treated with immunosuppressive agents and patients with IBD not receiving these agents.

METHODS: A systematic literature review was conducted, and a meta-analysis was performed on data retrieved from cohort studies that followed patients with IBD who received immunosuppressive agents for more than a year and documented the incidence of newly developed malignancy. An electronic search was conducted using MEDLINE (1966–September 2006), the Cochrane Library (issue 3, 2006), and Japana Centra Revuo Medicina (1981–September 2006). Medical subject headings used in the searches were azathioprine, 6-mercaptopurine, cyclosporine, methotrexate, tacrolimus, inflammatory bowel disease, and neoplasms. We imposed no language limitation in the searches. Additionally, a manual search of reference listings from all articles retrieved from the electronic databases was performed. Using data obtained from control groups or population-based studies, the incidence of newly developed malignancy in patients with IBD treated with immunosuppressive agents was compared with that of patients with IBD who were not receiving immunosuppressive agents. Statistical analysis for the change in risk of developing malignancy was performed using the weighted mean difference (WMD) normalized to per person-year and its 95% confidence interval.

RESULTS: Nine cohort studies met the inclusion criteria for this meta-analysis. Analysis of these studies showed no discernible difference (WMD -0.3×10^{-3}/person-year; 95% CI -1.2×10^{-3} to 0.7×10^{-3}) in the incidence of any kind of malignancy in patients with IBD who received immunosuppressants compared with those who did not receive immunosuppressants. No significant difference in WMD was observed when the data from patients with either Crohn's disease (CD) or ulcerative colitis (UC) were analyzed separately.

CONCLUSIONS: Our findings suggest that the administration of immunosuppressive agents in patients with either CD or UC probably does not confer a significantly increased risk of malignancy compared with patients with IBD who are not receiving these agents.

KEY WORDS: azathioprine, inflammatory bowel disease, malignancy, 6-mercaptopurine.

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Sulfasalazine and corticosteroids are the mainstay of pharmacotherapy for mild-to-moderate inflammatory bowel disease (IBD). However, immunosuppressive agents (eg, azathioprine and its metabolite, 6-mercaptopurine) are frequently supplemented with the above 2 agents, especially for patients resistant to or dependent on corticosteroids. Since patients with IBD tend to experience a protracted clinical course, they often receive substantial cumulative amounts of immunosuppressants.

Allergic reactions that develop shortly after initiation of immunosuppressive agent therapy are unpleasant and potentially harmful.[1-3] In addition, more serious concern has been raised as to whether long-term administration of these drugs may increase the risk of malignancy in patients with IBD. A recent meta-analysis suggested that patients with IBD who receive long-term immunosuppressive therapy with azathioprine or 6-mercaptopurine may experience a fourfold increase in risk of lymphoma relative to the general population.[4] This appears to be supported by findings from previous studies in patients who underwent prolonged immunosuppressive therapy due to organ transplantation or steroid-resistant rheumatoid arthritis.[5-12] However, caution should be exercised in interpretation of these data, because patients with IBD may possess an increased risk of lymphoma and other

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malignancies relative to the general population that is not related to treatment.[13-17]

To our knowledge, few attempts have been made to clarify whether immunosuppressive therapy for the treatment of IBD is associated with an increased risk of malignancies, including colorectal cancer. With this background, we undertook a systematic literature review and meta-analysis of this issue.

Methods

DATA SOURCES

To identify relevant cohort studies, an electronic search was conducted using MEDLINE (1966–September 2006), the Cochrane Library (issue 3, 2006), and Japana Centra Revuo Medicina (1981–September 2006). Medical subject headings used in the searches were azathioprine, 6-mercaptopurine, cyclosporine, methotrexate, tacrolimus, inflammatory bowel diseases and neoplasms. We imposed no language limitation in the searches. Additionally, a manual search of reference listings from all of the articles retrieved from the electronic databases was performed.

INCLUSION CRITERIA

The inclusion criteria of articles were that the studies: (1) were designed as cohort studies, (2) had a follow-up period of at least one year, (3) included patients with IBD given one of the immunosuppressive agents (ie, azathioprine, 6-mercaptopurine, cyclosporine, methotrexate, tacrolimus), (4) were designed to detect development of malignancy as a predetermined endpoint, and (5) provided information on the duration of immunosuppressive therapy. Three investigators (YM, RO, KO) applied the inclusion criteria independently. When disagreements occurred, all 3 conferred to arrive at a consensus.

ASSESSMENT OF LITERATURE QUALITY

The quality of the retrieved studies was assessed using a scoring system according to Downs and Black.[18] The system consists of 27 items grouped into 5 categories: reporting (10 items), external validity (3 items), internal validity-bias (7 items), internal validity-confounding (6 items), and power (1 item). Most of the items were scored either 0 or 1 according to the criterion specific to each item. However, items of reporting were scored 0, 1, or 2 and the item of power was scored from 0 to 5. Each study was evaluated according to the sum of the scores for all items. The quality was assessed as high, moderate, and low when the total scores were 20 or greater, from 10 to 19, and less than 10, respectively. Only studies of high or moderate quality were included in the meta-analysis. The 3 investigators scored the studies independently. When there was discordance among the quality scores reported by the 3 investigators, the lowest score was adopted.

DATA EXTRACTION

The investigators independently extracted the following data from the relevant cohort studies: design, number of subjects, use of immunosuppressive agents (ie, drug, daily dose, duration), length of follow-up, country in which the study was performed, study period, and the incidence and organ-specific carcinogenicity that developed in patients who participated in the studies.

DATA ANALYSES

Since the incidence of malignancy is assumed to be related to the number of patients and the length of the follow-up period, the incidence of developing malignancy in each study was transformed to events per person-year.

The risk of developing malignancy was compared between patients with IBD who received immunosuppressive agents and those who did not. In addition, analyses were performed separately for patients having either Crohn's disease (CD) or ulcerative colitis (UC). When possible, meta-analyses were performed to determine the risks of developing different types of malignancy (eg, colorectal cancer, malignant melanoma, leukemia, lymphoma) in patients with IBD.

For studies that had no control group (ie, patients with IBD who were followed but received no immunosuppressive agents), the incidence of malignancy for patients with CD, UC, or IBD, obtained from a population-based study performed in Manitoba, Canada, was used as the control data.[19] In the Manitoba IBD population study conducted in 1997, only approximately 8% of the patients were documented as receiving immunosuppressive agents (including azathioprine, 6-mercaptopurine, cyclosporine, methotrexate).[20] We chose the above data as control because previous studies that compared the incidence of malignancy between patients with IBD who received immunosuppressive therapy and the general population may have led to a biased conclusion.

The risk of developing malignancy in patients with IBD receiving immunosuppressive therapy relative to those receiving pharmacotherapy without immunosuppressive agents was calculated using a general variance-based method.[21] The data are expressed as the weighted mean difference (WMD) with the 95% CI. We adopted WMD rather than odds ratio because WMD represents the actual difference in incidence of an outcome parameter (ie, incidence of malignancy in person-year), thus giving a more realistic picture regarding the impact of the risk factor on the clinically observable outcome. A positive value of the pooled WMD indicates a greater risk of developing malig-

nancy in patients who received immunosuppressive therapy relative to those who did not, whereas a negative value indicates the opposite. The CIs were calculated assuming Poisson distribution for the events. Statistical significance between groups was evaluated using the 95% CI. Apart from WMD, the data were analyzed in terms of odds ratio with 95% CI.

For the meta-analysis of the retrieved studies, the heterogeneity among the studies was assessed using the Q statistics, where a p value less than 0.05 was considered significant. When no significant heterogeneity was observed regarding the risk estimates among the studies, the fixed-effects model was applied for further analysis.[21] When significant heterogeneity was observed, the random-effects model was applied.[21] Calculations of pooled WMDs with 95% CIs, as well as odds ratios with 95% CIs, were performed with Morizane software.[22]

Results

Among the 187 studies retrieved from the electronic databases and those retrieved from their references by manual search, we identified 9 cohort studies that satisfied the predetermined inclusion criteria for our study (Table 1).[23-31] While a language limitation for our systematic search was not set, all relevant articles that met our inclusion criteria were published in English.

The quality of each article was evaluated according to the scoring system by Downs and Black[18] (Table 1). All 9 cohort studies were assessed as high (ie, ≥20 points) or moderate (10–19 points) quality. Therefore, all 9 studies were included in our meta-analysis. Among the 4039 patients evaluated, 118 developed malignancy. When more than 2 immunosuppressive agents were employed, the incidence of malignancy for each agent was not reported.[26] Furthermore, no study included patients receiving tacrolimus. Thus, we analyzed the risk of developing malignancy in patients with IBD in terms of immunosuppressive agents in general.

Three of the 9 cohort studies[23,24,26] included appropriate control groups, while the other 6 studies did not.[25,27-31] For the 6 uncontrolled studies, the incidence of malignancy in patients with CD, UC, or IBD was calculated from a population-based study performed in Manitoba, Canada,[19] and used as control data (ie, patients not receiving an immunosuppressive agent).

As shown in Figure 1, the pooled WMD of the incidence of malignancy in patients with IBD who received one of the immunosuppressive agents versus that for control patients with IBD who received no immunosuppressant was -0.3×10^{-3}/person-year (95% CI -1.2×10^{-3} to 0.7×10^{-3}). We found no significant relationship between the length of exposure to immunosuppressive therapy and WMD in the incidence of development of malignancy (data not shown). Figure 2 shows results of pooled WMD in analysis that was performed separately for patients with CD or UC. Thus, there was no significant difference in the risk of malignancy regardless of the administration of immunosuppressants in patients with either CD or UC.

Analysis of the data with respect to odds ratio gave results that were essentially similar to those described above (data not shown).

Types of malignancy that were reported in the studies included colorectal cancer, malignant melanoma, leukemia, and lymphoma. Figure 3 shows the results for the meta-

Table 1. Summary of Studies Included in the Meta-Analysis

Reference	Disease	Age, y (mean)	Drug	Subjects, N (male/female)	Dosage	Treatment Duration, y	Follow-up Duration, y	Country	Literature Quality Score
Matula (2005)[23]	UC	29	AZA, 6-MP	96 (53/43)	60.6 mg[a]	7.4	8	US	20
Fraser (2002)[24]	IBD	32	AZA	626	NA	6.9	13.5	UK	18
Lewis (2001)[25]	CD, UC	41	AZA, 6-MP	1465	106 mg[b]	2	2.5	UK	20
Farrell (2000)[26]	IBD	40	AZA	212[c]	2–2.5 mg/kg	1.8	6.9	Ireland	19
			CyA	22[c]	2–4 mg/kg				
			MTX	31[c]	5 mg 3 times/wk				
Korelitz (1999)[27]	CD, UC	32	6-MP	550	50–75 mg	5.0	17.4	US	15
Bouhnik (1996)[28]	CD	31	AZA	157 (68/89)	2 mg/kg	2.1	2.6	France	18
			6-MP		1.5 mg/kg				
George (1996)[29]	UC	39	6-MP	105 (58/47)	50 mg	3.5	5.0	US	15
Connell (1994)[30]	CD, UC	NA	AZA	755[d] (389/366)	2 mg/kg	1.0	9.0	UK	17
Lobo (1990)[31]	UC	38	AZA	47 (23/24)	1.9 ± 0.3 mg/kg	1.0	1.8	UK	14

AZA = azathioprine; CD = Crohn's disease; CyA = cyclosporine; IBD = inflammatory bowel disease; 6-MP = 6-mercaptopurine; MTX = methotrexate; NA = not available; UC = ulcerative colitis.

[a]The dose of azathioprine was converted to 6-mercaptopurine equivalent.

[b]The dose of 6-mercaptopurine was converted to azathioprine equivalent.

[c]A total of 238 patients with inflammatory bowel disease received immunosuppressive agents.

[d]Comprised of 450 patients with CD, 282 patients with UC, and 23 patients with indeterminate colitis.

analyses that were performed separately for the risks of developing different types of malignancy. None of the values was considered significant.

Discussion

For patients with IBD who are resistant to or dependent on corticosteroids, current guidelines recommend one of the immunosuppressants (eg, azathioprine or 6-mercaptopurine) as an adjunctive therapeutic agent.[32-34] Since previous studies revealed that long-term administration of these agents in patients undergoing renal transplantation[5-7] or in patients with rheumatoid arthritis[8-12] conferred an increased risk of developing malignancy, a concern has been raised regarding the possible harm of current therapeutic strategies for patients with severe IBD. Using systematic literature review combined with meta-analysis, our study demonstrated that these immunosuppressive agents administered to patients with IBD do not significantly increase the risk of malignancy. The results of our study have substantial clinical implications; however, our findings cannot substitute for those of a large-scale, prospective, placebo-controlled trial.

There are fundamental difficulties in assessing a possible relationship between any therapeutic measure and altered risk of malignancy in patients with IBD. First, this population is known to be at increased risk for colorectal cancer compared with the general population. More specifically, the overall prevalence of colorectal cancer in patients with UC has been estimated to be 3.7% (95% CI 3.2 to 4.2).[35] Similarly, the corresponding rates in patients with UC at 10, 20, and 30 years from disease onset were reported to be 2%, 8%, and 18%, respectively. Previous population-based studies of the risk for colorectal cancer have shown that patients with CD have standardized incidence ratios of 1.39–2.64 relative to the general population.[19,36-38] In addition, since the number of patients studied in previous therapeutic trials is relatively small, each study was underpowered to identify small increases in the risk of malignancy. In this context, our meta-analysis is an attractive alternative approach to shed light on this issue.

Since patients with CD and those with UC may have different risks for development of malignancy, in particular colorectal cancer, it would be prudent to analyze the data obtained from each group separately. Our results indicated that there is no discernible trend toward an increased risk of malignancy, not only in patients with CD but also in those with UC (Figure 2). It is of interest to investigate whether immunosuppressive therapy for IBD is associated with specific type(s) of malignancy, because cyclosporine therapy in patients undergoing renal transplantation has been associated with increased risk of cutaneous malignancy (eg, malignant melanoma) and, to a lesser extent, with non-Hodgkin's lymphoma and other solid tumors.[5] In addition, immunosuppressive therapy (mainly azathioprine and cyclophosphamide) in patients with rheumatoid arthritis was proposed to be associated with an increased risk of lymphoma.[8-10] To address this issue, we performed meta-analyses for different types of malignancy (Figure 3). Our results indicated no discernible changes in the risks of de-

	IBD Patients					
	Immunosuppressive Agents		No Immunosuppressive Agents (Control Group)			
Reference	N	Incidence of Developing Malignancy (± SD) × 10^{-3}/person-year	N	Incidence of Developing Malignancy (± SD) × 10^{-3}/person-year	Immunosuppressive Agents ←Risk (–) Risk (+)→	WMD, fixed-effects (95% CI) × 10^{-3}/person-year
Matula (2005)[23]	96	6.4 (80.2)	219	6.4 (79.7)		0.0 (–19.2, 19.2)
Fraser (2002)[24]	626	7.2 (84.7)	1578	3.6 (59.7)		3.6 (–3.6, 10.9)
Lewis (2001)[25]	1465	0.2 (15.4)	5529[a]	0.4 (19.8)[b]		–0.2 (–1.1, 0.8)
Farrell (2000)[26]	238	8.5 (92.3)	544	3.7 (60.6)		4.9 (–7.9, 17.6)
Korelitz (1999)[27]	550	2.7 (52.1)	5529[a]	6.9 (83.1)		–4.2 (–9.1, 0.7)
Bouhnik (1996)[28]	157	9.9 (99.3)	2857[a]	6.1 (78.4)		3.7 (–12.1, 19.5)
George (1996)[29]	105	11.4 (106.9)	2672[a]	7.7 (87.9)		3.7 (–17.0, 24.4)
Connell (1994)[30]	755	4.6 (67.5)	5529[a]	6.9 (83.1)		–2.3 (–7.6, 3.0)
Lobo (1990)[31]	47	11.6 (107.8)	2672[a]	7.7 (87.9)		3.9 (–27.1, 34.9)
TOTAL	4039		27 129			–0.3 (–1.2, 0.7)

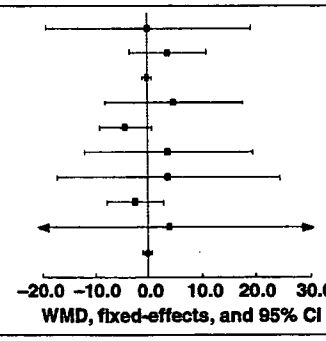

Figure 1. Meta-analysis of 9 studies on the incidence of developing malignancy in patients with inflammatory bowel disease who received immunosuppressive agents compared with those not treated with an immunosuppressant. Data in the diagram are the weighted mean differences (WMD; event 10^{-3}/person-year) with 95% confidence intervals. A positive value for WMD is associated with an increased risk of the development of malignancy in patients who received immunosuppressive agents. The analysis was performed using the fixed-effects model.
IBD = inflammatory bowel disease; WMD = weighted mean difference.
[a]Number of patients whose data were derived from a Canadian population-based study.[19]
[b]The incidence of lymphoma obtained from the population-based study[19] is used for comparison since Lewis et al.[25] reported the incidence of lymphoma only. For other studies, the incidence of all types of malignancy is shown.

veloping colorectal cancer, malignant melanoma, leukemia, or lymphoma, regardless of the administration of immunosuppressive agents in patients with IBD.

Selection of the control population may constitute a point for debate. Six of the 9 studies retrieved from the literature lacked a parallel control group of patients with IBD not treated with immunosuppressive agents. In these studies, we had to employ a comparable control group of patients with IBD not treated with immunosuppressants and with documented risk of malignancy. We used data from a population-based study conducted in Canada,[19] in which only approximately 8% of the patients received immunosuppressive agents (ie, azathioprine, 6-mercaptopurine, cyclosporine, or methotrexate).[20] The rationale for selecting the subjects in this study to serve as the control population was that the ethnic backgrounds and geographic environment were largely comparable with those of the subjects included in our meta-analysis.

While the incidence rates of UC and CD in the US are 11 and 7 per 100 000 persons, respectively, those in southern Europe, South Africa, and Australia are 2–6.3 and 0.9 –3.1 per 100 000, and those in Asia and South America are 0.5 and 0.08 per 100 000, respectively.[39] The 9 cohort studies included in our meta-analysis were performed in Europe or the US. In contrast, Kandiel et al.[4] used the data obtained from the general population as control, because they assumed that the risk of lymphoma in patients with IBD does not differ significantly from that of the general population. The difference in the choice of control for the meta-analysis would explain, at least partially, why there is a contradiction between our findings and those of Kandiel et al. regarding the risk of lymphoma associated with immunosuppressive therapy in patients with IBD.

Recent studies cast doubt on the cost-effectiveness of colonoscopic surveillance in patients with long-standing and extensive UC who are assumed to possess an increased risk of colorectal cancer.[40,41] Since our meta-analysis showed that administration of immunosuppressive agents would not significantly increase the risk of various malignancies, including colorectal cancer, in patients with IBD compared with patients with IBD who receive no immunosuppressive agents, colonoscopic surveillance of patients with UC who are receiving immunosuppressive agents may not be recommended.

Limitations

Although systematic literature review coupled with meta-analysis is a powerful technique for analyzing the causal relationship between the administration of drugs and rare clinical events during follow-up periods, caution must be exercised in interpreting the results. Since this method relies on retrospective data collection, protocols of the retrieved studies are not necessarily the same in terms

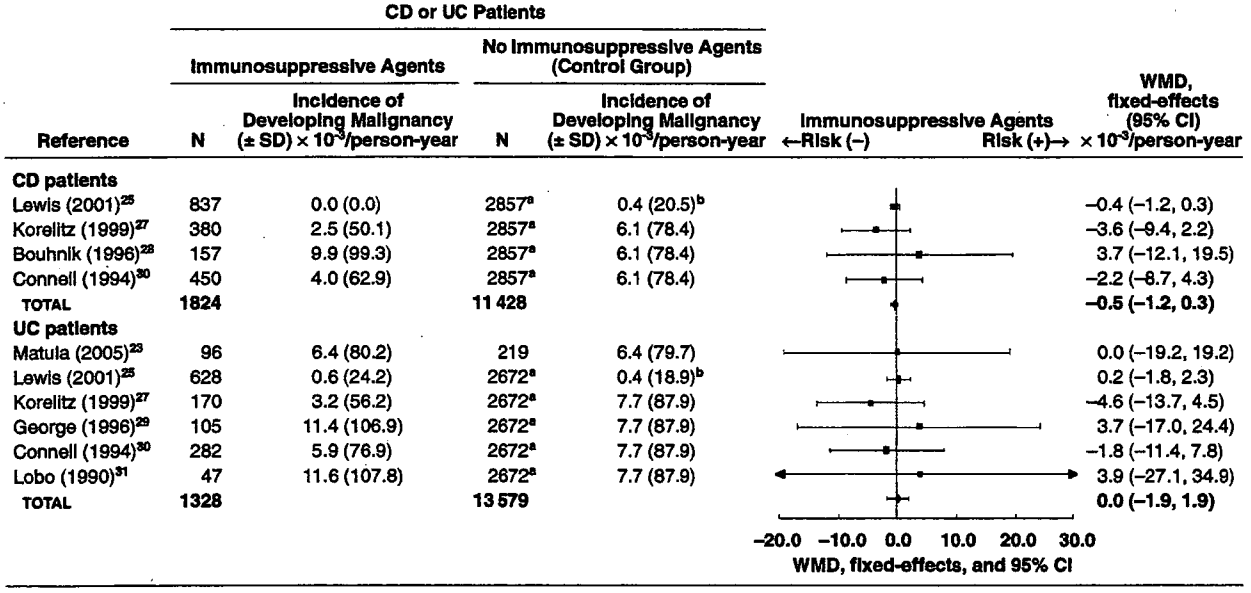

Figure 2. Meta-analysis of studies on the incidence of developing malignancy in patients with Crohn's disease or ulcerative colitis who received immunosuppressive agents compared with those not treated with an immunosuppressant. Data in the diagram are the weighted mean differences (WMD; event 10^{-3}/person-year) with 95% confidence intervals. A positive value for WMD is associated with an increased risk of the development of malignancy in patients who received immunosuppressive agents. The analysis was performed using the fixed-effects model.
CD = Crohn's disease; UC = ulcerative colitis; WMD = weighted mean difference.
[a]Number of patients whose data were derived from a Canadian population-based study.[19]
[b]The incidence of lymphoma obtained from the population-based study[19] is used for comparison since Lewis et al.[25] reported the incidence of lymphoma only. For other studies, the incidence of all types of malignancy is shown.

of the doses of drugs and study periods. For instance, while the daily doses of azathioprine (2.0–2.5 mg/kg or 106 mg/day) or 6-mercaptopurine (1.5 mg/kg or 50–75 mg/day) were largely comparable among the studies, the length of immunosuppressive therapy differed substantially among the studies (1.0–7.4 y). To circumvent this problem, we employed a time-independent index of risk assessment (ie, person-year), assuming that the effect of the drugs on the risk of malignancy is cumulative and increases linearly with duration of the immunosuppressive therapy for patients with IBD. While this is a likely premise, it has not been proven. Moreover, patient adherence to pharmacotherapy would have a major impact on the outcomes of cohort studies. It is nearly impossible to verify adherence in any of the studies included in our meta-analysis unless definitive descriptions were provided in the original articles.

Conclusions

The findings of our meta-analysis suggest that administration of immunosuppressive agents probably does not increase the risk of malignancy in patients with CD or UC compared with patients with CD or UC who are not receiving immunosuppressants. Our findings do not support the concern about the possible risk of carcinogenicity of immunosuppressive therapy frequently employed in patients with severe IBD who are resistant to or dependent on corticosteroids.

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	IBD Patients					
	Immunosuppressive Agents		No Immunosuppressive Agents (Control Group)			
Reference	N	Incidence of Developing Malignancy (± SD) × 10^{-3}/person-year	N	Incidence of Developing Malignancy (± SD) × 10^{-3}/person-year	Immunosuppressive Agents ←Risk (–) Risk (+)→	WMD, fixed-effects (95% CI) × 10^{-3}/person-year
Colorectal cancer						
Matula (2005)[23]	96	6.4 (80.2)	219	6.4 (79.7)		0.0 (–19.2, 19.2)
Fraser (2002)[24]	626	1.6 (40.3)	1578	1.1 (32.6)		0.6 (–3.0, 4.1)
Farrell (2000)[26]	238	3.7 (60.5)	544	2.1 (45.5)		1.6 (–7.0, 10.2)
Korelitz (1999)[27]	550	0.5 (23.3)	5529[a]	1.9 (46.6)		–1.4 (–3.7, 0.9)
George (1996)[29]	105	5.7 (75.6)	2672[a]	2.5 (50.0)		3.2 (–11.4, 17.8)
Connell (1994)[30]	755	1.9 (43.7)	5529[a]	1.9 (43.6)		0.0 (–3.2, 3.3)
TOTAL	**2370**		**16 071**			**–0.5 (–2.1, 1.2)**
Malignant melonoma						
Farrell (2000)[26]	238	0.6 (24.7)	544	0.0 (0.0)		0.6 (–2.5, 3.8)
Korelitz (1999)[27]	550	0.1 (10.4)	5529[a]	0.2 (13.1)		–0.1 (–1.0, 0.9)
Bouhnik (1996)[28]	157	2.5 (49.7)	2857[a]	0.1 (11.9)		2.3 (–5.5, 10.1)
TOTAL	**945**		**8930**			**0.0 (–0.9, 0.9)**
Leukemia						
Fraser (2002)[24]	626	0.0 (0.0)	1578	0.1 (6.8)		–0.1 (–0.4, 0.3)
Korelitz (1999)[27]	550	0.1 (10.4)	5529[a]	0.2 (13.1)		–0.1 (–1.0, 0.9)
Connell (1994)[30]	755	0.2 (12.1)	5529[a]	0.2 (13.1)		0.0 (–1.0, 0.9)
Lobo (1990)[31]	47	11.6 (107.8)	2672[a]	0.2 (14.3)		11.4 (–19.4, 42.3)
TOTAL	**1978**		**15 308**			**–0.1 (–0.4, 0.3)**
Lymphoma						
Fraser (2002)[24]	626	0.7 (26.4)	1578	0.2 (15.2)		0.5 (–1.7, 2.7)
Lewis (2001)[25]	1465	0.2 (15.4)	5529[a]	0.4 (19.8)		–0.2 (–1.1, 0.8)
Farrell (2000)[26]	238	2.4 (49.4)	544	0.0 (0.0)		2.4 (–3.8, 8.7)
Korelitz (1999)[27]	550	0.2 (14.7)	5529[a]	0.4 (19.8)		–0.2 (–1.5, 1.2)
Bouhnik (1996)[28]	157	2.5 (49.7)	2857[a]	0.4 (20.5)		2.1 (–5.8, 9.9)
Connell (1994)[30]	755	0.0 (0.0)	5529[a]	0.4 (19.8)		–0.4 (–0.9, 0.1)
TOTAL	**3791**		**21 566**			**0.0 (–0.8, 0.7)**

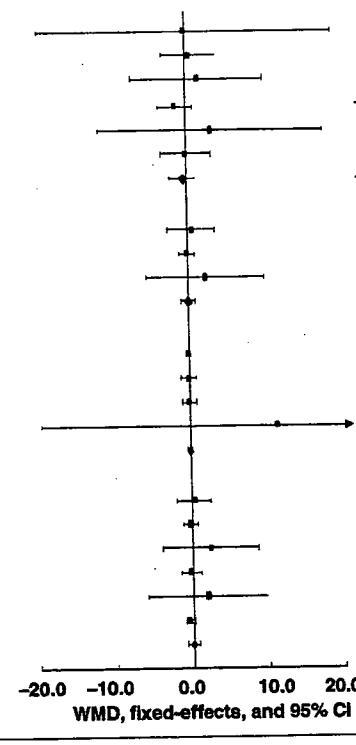

Figure 3. Meta-analysis of studies regarding the incidence of developing malignancy in patients with inflammatory bowel disease who received immunosuppressive agents compared with those not treated with an immunosuppressant. Data in the diagram are the weighted mean differences (WMD; event 10^{-3}/person-year) with 95% confidence intervals. A positive value for WMD is associated with an increased risk of the development of malignancy in patients who received immunosuppressive agents. The analysis was performed using the fixed-effects model.
IBD = inflammatory bowel disease; WMD = weighted mean difference.
[a]Number of patients whose data were derived from a Canadian population-based study.[19]

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EXTRACTO

TRASFONDO: Existe la preocupación de si la administración de inmunosupresores por períodos largos en pacientes con enfermedad inflamatoria intestinal (EII) pudiera aumentar el riesgo de malignidad.

OBJETIVO: Comparar los riesgos de desarrollar malignidad entre los pacientes con EII tratados con agentes inmunosupresores y en pacientes con esta enfermedad que no reciben terapia con dichos agentes.

MÉTODOS: Se llevó a cabo una revisión sistemática de la literatura junto con un meta análisis de los datos recuperados de estudios de cohorte que dieron seguimiento a pacientes con EII por más de un año y que documentaron la incidencia de malignidad recién desarrollada. Se comparó la incidencia con la de los pacientes que no recibieron agentes inmunosupresores obtenida de grupos control o de estudios basados en población. Se recuperó información hasta septiembre de 2006 de las bases de datos (p.ej., MEDLINE y Cochrane Library) utilizadas. Se llevó a cabo un análisis estadístico sobre el cambio en el riesgo de desarrollar malignidad utilizando la diferencia media estandarizada (DME) en personas-año y su intervalo de confianza de 95%.

RESULTADOS: Nueve estudios de cohorte cumplieron los criterios de inclusión para el meta análisis. Los análisis de estos estudios no demostraron diferencias discernibles (DME -0.3 [de –1.2 a 0.7] × 10^{-3}/personas-año) en la incidencia de cualquier tipo de malignidad en pacientes con EII que recibieron inmunosupresores en comparación con pacientes con EII que no reciben inmunosupresores. No se observó diferencia significativa en la DME cuando los datos de los pacientes con enfermedad de Crohn (EC) o colitis ulcerativa (CU) fueron analizados separadamente.

CONCLUSIONES: Este estudio sugiere que no es probable que la administración de agentes inmunosupresores en pacientes con EC o CU confiera un aumento significativo en el riesgo de malignidad en comparación con los pacientes con EII que no reciben estos agentes.

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RÉSUMÉ

CONTEXTE: Il y a une controverse à propos de l'augmentation éventuelle du risque de malignités lors de l'administration au long cours d'immunosuppresseurs à des patients atteints de maladies inflammatoires intestinales (MII).

OBJECTIFS: Comparer les risques de développer des malignités chez des patients atteints de MII traités par immunosuppresseurs et chez des patients atteints de MII ne recevant pas ces traitements.

MÉTHODES: Il a été conduit une revue systématique de la littérature couplée à une méta-analyse sur les données extraites des études de cohorte qui ont suivi des patients atteints de MII recevant des immunosuppresseurs pendant plus d'un an et qui ont documenté l'incidence de développement de nouvelles malignités. L'incidence a été comparée avec celle de patients atteints de MII ne recevant pas d'immunosuppresseurs, obtenue à partir de groupes témoins ou, à défaut, d'une étude de population. Les données ont été extraites de banques de données électroniques (par ex.: MEDLINE et The Cochrane Library) jusqu'en septembre 2006. L'analyse statistique pour les modifications du risque de développer des malignités a été conduite au moyen de la différence moyenne pondérée (DMP) standardisée par personne-année et son intervalle de confiance à 95%.

RESULTATS: Neuf études de cohorte remplissaient les critères d'inclusion de la méta-analyse. L'analyse de ces études n'a pas montré de différence perceptible (DMP -0.3 [–1.2 à 0.7] × 10^{-3}/personne-année) en ce qui concerne l'incidence d'un quelconque type de malignité chez les patients atteints de MII qui recevaient des immunosuppresseurs comparée à celle des patients atteints de MII ne recevant pas d'immunosuppresseurs. Aucune différence significative sur la DMP n'a été observée lorsque les données des patients atteints de maladie de Crohn ou de rectocolite hémorragique (RCH) étaient analysées séparément.

CONCLUSIONS: Cette étude suggère que l'administration d'immunosuppresseurs à des patients atteints de maladie de Crohn ou de RCH n'entraîne vraisemblablement pas de risque significativement accru de malignité par rapport à des patients atteints de MII ne recevant pas ce type de médicaments.

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