Changes Associated With Lenalidomide Treatment in the Gene Expression Profiles of Patients With Del(5q)

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Abstract

We used microarray profiling to investigate the direct effects of lenalidomide on gene expression in isolated CD14$^+$ monocytes from 6 patients with del(5q). Our data demonstrate that changes in genes involved the tumor necrosis factor (TNF) signaling pathway and the bone marrow stroma, suggesting that treatment with lenalidomide may help restore the damaged niche and suppress the TNF signaling pathway.

Background: Lenalidomide is an effective treatment for patients with del(5q) and myelodysplastic syndrome (MDS) The exact mechanism of lenalidomide function and its impact on the prognosis of patients is not known exactly. **Materials and Methods:** We used gene expression profiling to study the effect of lenalidomide therapy in peripheral blood CD14$^+$ monocytes of 6 patients with del(5q) and MDS. **Results:** After lenalidomide treatment, genes involved in the tumor necrosis factor (TNF) signaling pathway that were upregulated in the patients before treatment decreased to the healthy control baseline expression level. This change in gene expression, in conjunction with increased expression of repressed genes that affect the stem cell niche (ie, *CXCR4* and *CRTAP*), may exert a positive effect on treated patients. In contrast, we found that increased expression of the *ARPC1B* gene may have a negative impact on the stability of patient remission. **Conclusion:** The observed changes in gene expression described here may contribute to the identification of pathways that are affected by lenalidomide, which may help to explain the effects of this drug.

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Introduction

Interstitial del(5q) is 1 of the most common structural rearrangements present in myelodysplastic syndrome (MDS). Boultwood et al[1] described a 1.5-megabase interval that contains 40 genes as the common deleted region (CDR). Patients with low- and intermediate-1-risk del(5q) MDS respond well to lenalidomide and have a 56% independence from transfusion and a 50% complete cytogenetic response.[2] However other studies have shown that patients with del(5q) who fail to achieve a sustained erythroid or cytogenetic remission after lenalidomide treatment have an increased risk of clonal evolution and acute myeloid leukemia (AML) progression.[3,4,5]

Lenalidomide, which is a thalidomide analogue, was approved by the US Food and Drug Administration in 2005 for the treatment of patients with del(5q) and MDS. Lenalidomide is an immunomodulatory drug that affects the cellular and humoral branches of the immune system.[6] The exact mechanism of lenalidomide function and its impact on prognosis in patients del(5q) and MDS is unknown. However in vitro studies have shown that lenalidomide downregulates the production of the proinflammatory cytokines tumor necrosis factor alpha (TNF-α), interleu-

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Table 1 Patient Characteristics

Patient No.	Sex	Age (y)	Karyotype at Baseline	Interphase FISH Del(5)(q31) at Baseline (%)	Karyotype at 24 wk	Interphase FISH Del(5)(q31) at 24 wk (%)	Transfusions at Baseline	Transfusions at 24 wk
1	M	63	46,XY,t(2;11) (p21;q23),Del(5) (q13.3q33.3)[20]/46XY[2]	NA	46,XY,t(2;11) (p21;q23),del(5) (q13.3q33.3)[4]/46XY[12]	70	Yes	Yes
2	F	75	46,XX,del(5) (q13.3q33.3)[9]/46,XX[11]	64	46,XX[19]	0	Yes	No
3	F	59	46,XX,del(5) (q13.3q33.3)[1]/46,XX[4]	49.5	46,XX[22]	NA	Yes	No
4	M	73	46,XY,Del(5) (q13.3q33.3)[7]/46,XY[7]	74.5	46,XY,del(5) (q13.3q33.3)[3]/ 46,XY[16]	NA	Yes	No
5	M	55	46,XY[15]	54	46,XY,der(2) t(2;5) (p?13; q33),der(5)del(5) (q13.2q33.3)t(2;5)(p?13; q33.3)[7]/46,XY[9]	46	Yes	No
6	F	72	46,XX,del(5) (q31)[10]/ 46,XX[5]	64.5	46,XX,del(5) (q31)[4]/46,XX[18]	NA	Yes	No
7	F	63	No mitosis	36.5	46,XX,del(5) (q31)[2]/46,XX[20]	9	Yes	No
8	F	73	46,XX[15]	66	46,XX[22]	0	Yes	No

Abbreviations: Interphase FISH = x-fluorescence in situ hybridization; NA = not applicable.

kin-1β (IL-1β), and transforming growth factor beta-1 (TGF-β1) by activated monocytes.[7,8]

In this study we used expression profiling to investigate the direct effects of lenalidomide on gene expression in isolated CD14$^+$ monocytes from patients with del(5q). Moreover, we monitored the expression levels of selected genes during the treatment course.

Materials and Methods

Samples

The study population included 7 patients (4 women and 3 men) with del(5q) MDS who were treated with lenalidomide. Samples 1 to 6 were used for profiling, and samples 1, 2, and 4 to 7 were used for real-time polymerase chain reaction (PCR). Patient age ranged from 55 to 75 years (median, 68 years). Peripheral blood (PB) samples were collected from the patients before and during treatment. The clinical characteristics of the patients are summarized in Table 1. Lenalidomide was administered as recommended at 10 mg/d for 21 days with a 1-week interruption. The starting dose of 10 mg was reduced to 5 mg if there was any sign of bone marrow (BM) toxicity such as thrombocytopenia.

PB samples were collected from 10 healthy donors (6 for gene expression profiling and 10 for quantitative real-time PCR) were used as controls (median age, 60 years). All of the patients gave informed consent, and this study was approved by an institutional review board.

CD14$^+$ Cell Separation and RNA Isolation. CD14$^+$ monocytes were isolated from the patient and control PB samples. The mononuclear cells were purified with Ficoll-Hypaque density centrifugation (GE Healthcare, Piscataway, NJ), and the CD14$^+$ cells were isolated with a magnetic separation kit (magnetic-activated cell sorting [MACS]; Miltenyi Biotec, Cologne, Germany). The acid guanidinium-thiocyanate-phenol-chloroform method was used to extract the total RNA.[9]

Conventional Karyotyping. The unstimulated bone marrow cells were cultivated for 24 hours in RPMI 1640 medium with 10% fetal calf serum. The chromosomal preparations were performed according to standard techniques with colcemid (which is a hypotonic treatment), fixation in methanol/acetic acid, and G-banding with Wright-Giemsa stain. The karyotypes were described according to the International System of Human Cytogenetic Nomenclature (ISCN 2009).[10]

Fluorescence in Situ Hybridization. A Vysis LSI EGR1/D5S23,D5S721 Dual Color Probe (Abbott Molecular, Abbott Park, IL) was used to confirm a genetic deletion in the 5q31 region. The fluorescence in situ hybridization (FISH) assays were performed according to the manufacturer's protocols. At least 200 interphase nuclei were analyzed using a fluorescent motorized Axioplan II imaging microscope (Carl Zeiss, New York, NY) by 2 independent observers. The cutoff level was established at 5% based on the analyses of bone marrow samples from 10 healthy controls.

Gene expression profiling and data analysis. HumanRef-8 v3 Expression BeadChips (Illumina Inc, San Diego, CA) were used to generate expression profiles of 6 patients and 6 controls (> 24,000 probes). Patient samples from pretreatment and treatment at the time of the first erythroid response (2-5 months) were analyzed. The data from 1 patient after treatment were excluded because of a weak signal. The microarrays were processed according to the manufacturer's recommendations.

The chip scanning was performed with a BeadStation 500 instrument (Illumina Inc), and the raw data were extracted with the BeadStudio Data Analysis Software (Illumina). The R software lumi package was used to further process the raw data. After quantile normalization, the detected probes were filtered with a detection P value of $< .01$. A significance analysis of microarrays was performed