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研究室で撮影した本人のスナップ写真、及び発表論文のコピーを添付

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研究テーマ 転写抑制遺伝子GCFに特異的に結合する新しい蛋白質因子の構造と機能

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2．本年度の研究業績

(1) 学会・研究会等においての口頭発表 有 ・ (無) (学会名・内容)

(2) 学会誌等に発表した論文 (有) ・ 無 (雑誌名・論文名)

1.. "Revision of cDNA and protein structures of transcription factor GCF" Nucleic Acids Symposium Series No. 39 199-200

2. "Molecular Cloing of A Novel Human Protein that Binds Specifically to Nuclear Factor GCF" Nucleic Acids Symposium Series No. 39 201-202

3．今後の研究計画

We have cloned a novel human factor, D40, by two-hybrid system, but the function of D40 is still unknown. In order to understand the function of D40:

1. Spermotogenesis: Northern bloting shown that D40 is specifically transcripted in testis.

2. Tumor-specific antigen: The expression of D40 can be found in all tumor cell lines and several primary tumors.

4．研究指導者の意見

申請者衛　鋼は、大学院医学研究科博士課程に在学中で、すでに新しい転写調節因子を見つけるという大きな業績をあげています。日中医学学術交流制度のお陰で、この研究を深め、学業に専念することをできています。

申請者は熱心に勉強しているとともに、日中両国の交流など課外活動にも積極的に参加しています。本人は将来、日本での研究成果を母国の発展に活かし、国の発展と医学の進歩に貢献したいとの決意を強く固めています。

研究指導者氏名　高老遅 ㊞

5．研究報告

別紙形式を参考に、報告本文4000字以上で報告して下さい（枚数自由・ワープロ使用）
タイトル・要旨等は日本語で、KEY WORDS以下は日本語或いは英語で記入して下さい。
研究成果の発表予定がある場合は発表原稿・抄録集等を添付して下さい。
論文発表に当っては、日中医学協会－日本財団補助金による旨を明記して下さい。

転写制御因子GCFに特異的に結合するヒト細胞蛋白質D40の染色体マッピング

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「目的」我々は、D40が転写制御因子GCFに特異的に結合する新しいヒト蛋白質であることを報告してきた。今回、D40が癌を含む何らかの疾患に関与するか否かを検討するために、FISH法を用いてD40の染色体上の位置を決定することを試みた。

「方法と結果」染色体標本は正常人の抹消血リンパ球から作成した。プローブDNAとして、D40のcDNA全長にわたる複数のcDNAクローンを混合したものを用いた。これをビオチン標識したプローブとハイブリダイズした後、抗ビオチン抗体およびFITC抗ヤギ抗体を用いてシグナルを増幅した。これを蛍光顕微鏡にて観察した。その結果、ヒト第１５染色体14q13-15にマッピングされることがあきらかになつた。

「結論」転写制御因子GCF結合蛋白質D40は、白血病などで異常が多く報告されているヒト第１５染色体長腕に坐位する。

Key Words: Transcriptor, Chromosome

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The GC-factor (GCF) was firstly reported to be a transcriptional regulator that represses the transcription of the epidermal growth factor receptor (EGF-R) (1). It also regulates negatively expression of several other growth factor and receptor genes, including TGF-ā and IGF-1R (2,3). It was reported that GCF could inhibit the growth of human gastric carcinoma cells in nude mice due to decrease in TGF-a expression (2). GCF is a phosphoprotein and predominantly localized in the nucleus (4). For several transcription factors, phosphorylation increases nuclear transport (5). GCF phosphorylation may be involved in its nuclear translocation. Phosphorylation may serve to induce binding to other regulatory proteins, regulate DNA binding, or activate a constitutively bound transcription factor (6). Since a Leucine-zipper like sequence that could mediate protein-protein interactions was found in the middle of the coding region of GCF gene, we have sucessfully used yeast two-hybrid system to isolate the gene encoding GCF related proteins. After screenning of cDNA library derived from immortalized human B lymphocytes, a novel human cellular gene ,GCF-BP(D40), that encoding protein specifically binds to GCF was cloned. Northern blot analysis indicated the testis-specific expression of GCF-BP(D40) and high expression in spermatocytes *in situ* hybridization (manuscript in preparation). To gain further insight into the function and possible relation to human diseases , we here determined the chromosomal assignment of the GCF-BP (D40) gene by fluorescence *in situ* hybridization (FISH).

Lymphocytes isolated from bloods of normal adult male human were cultured in a-minimal essential medium (MEM) supplemented with 10% fetal calf serum and 3ug/ml Conchanavalin-A in 5% CO_2 at 37^{0}C for 40h. The cultured lymphocytes were treated with Bromodeoxyuridine (30ug/ml) for 7.5h at 37^{0}C. Colcemid was added during the incubation of

the final 30min (7). Cells were harvested and slides were prepared using standard procedures, including fixation and air drying.

For GCF-BP (D40) mapping, the five overlapped clones derived from colony hybridization that encods to the full length cDNA of GCF-BP (D40) were used as probe. Fluorescence *in situ* hybridization (FISH) on R-banded chromosomes was performed as previously described (8). In general, metaphase spreads were prepared from concanavalin-A stimulated lymphocytes of normal male human after bromodeoxyuridine incorporation. Two hundred nanograms of mixed DNA probe was nick-translated with biotin-16-dUTP (Boehringer Mannheim) and hybridized *in situ* to metaphase chromosomes in the denatured chromosome on slides. After hybridization at 37^{0}C for 48h , slides were washed in 50% formamide/0.5X SSC and 2X SSC sequentially. FISH signals were detected by sequential incubation with 3ug/ml anti-biotin goat IgG (Vector) and 40ug/ml FITC-conjugated anti-goat IgG (American Qualex). FITC signals were further amplified by the incubation with 40ug/ml anti-FITC goat IgG (Biomeda) and FITC-conjugated anti-goat IgG. After a screening under microscope to check the hybridization efficiency, FISH signals and DAPI-banded chromosomes were photographed seperately, and the assignment of FISH mapping data with chromosomal bands was obtained by superimposing FISH signals with DAPI-banded chromosomes.

Detailed analysis more than 10 individual chromosomes, the gene encoding GCF-BP (D40) maps to the chromosome 15q14-15. As shown in figure 1, fluorescence signals were detected mostly at the centromere region of chromosome 15. No any significant fluorescence signal was detected on other chromosomes. Chromosome 15 localization was further substantiated by simultaneous labeling with specific to the peri-centromeric region of chromosome 15. Intrestingly, cytogenetic observations and molecular studies have identified 15q11-13 as the critical regions for the Parder-Willi syndrome (PWS) and Angelman syndrome (AS) (9-10). The aberrations of chromosome 15q15 were observed in acute myeloid leukaemia (11). Recently, the tumor supressor activity of human chromosome 15 was reported (12). Thus, this report may provide new clues to the understanding of human chromosome 15 associated genetic disorders.

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