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学 位 論 文 名	Characterization of the sensitivity to various stress agents in Roberts syndrome lymphoblastoid cell lines （ロバーツ症候群患者のリンパ芽球のストレス薬剤に対する感受性に関する研究）
論文審査委員	（主査） 教　授　大薗　恵一 （副査） 教　授　吉川　秀樹　　教　授　戸田　達史

論 文 内 容 の 要 旨

〔 Aim 〕

To investigate the sensitiviry of RBS lymphoblastoid cell lines to a diverse set of stress agents including DNA damaging agents. To explore the cause underlying the hypersensitivity of RBS cells to DNA damaging and other stress agents

〔 Methods and Results 〕

EBV-inmortalized LCLs were established by infecting peripheral blood lymphocytes from either normal donors or RS patients with the EBV strain B95-8. Sensitivity to Mitomycin C (MMC), sodium orthovanadate (OV), G418, staurosporine, Okadaic acid and Camptothecin was studied by flow cytometry and fluorescein diacetate (FDA) staining. Apoptotic cells and cell cycle distribution were assayed using propidium iodide (PI) staining followed by flow cytometry analysis. Alternatively, cytospin slides were prepared and stained with May-Grünwald/Giemsa for morphological evaluation. P53 levels were analyzed by immunobloting with the anti-human p53 FL-393 antibody from Santa Cruz Biotechnology. Statistical comparisons among groups were made using one way analysis of variance (ANOVA). Post-hoc analysis based on Scheffe test using SPSS version 12. was used for comparison of individual cell lines mean values. For apoptosis, estimation of the difference between means was derived using the Student's paired t-test. A value of p less than 0.05 was considered statistically significant for all tests. We found that RBS cells are hypersensitive to MMC, G418, and OV while they present the same sensitivity as the normal cells in response to camptothecin, staurosporine and okadaic acid. We found that they underlying cause of RBS cells hypersensitivity to MMC, G418 and OV is an increased cell death. Our results also indicate that the mechanism of MMC-, G418- and OV- induced cell death in RBS LCLs appears to be through apoptosis. The analysis of cell cycle distribution after the MMC, G418 and OV treatment showed that cell cycle perturbations are induced exclusively by MMC. Cell cycle perturbations induced by low doses of MMC (0.3 mM) showed accumulation of cells in S phase at 18 to 24h and later a G2/M

arrest. Treatment with higher dosages (1.4 mM) resulted in S-phase arrest in both normal and RBS cells. The study of p53 levels showed that, 24 and 48 hr after orthovanadate treatment, p53 induction was greater in RBS cells. After MMC and G418 treatment, no differences in p53 levels were found between normal and RBS cell lines.

〔 Summary 〕

Hypersensitivity to DNA damaging agents has been useful in elucidating the role that genes mutated in chromosomal instability syndromes have in DNA damage sensing or repair pathways. Roberts syndrome (RBS) is a developmental disorder characterized by tetraphocomelia growth retardation and craniofacial abnormalities. Cells from RBS affected individuals exhibit premature separation of heterochromatic regions and cell cycle defects. We recently found that RBS is caused by mutations in ESCO2 gene, a human homolog of yeast ECO1/CTF7 that is essential for sister chromatid cohesion. It has been proposed that sister chromatid cohesion is involved not only in accurate segregation of chromosomes but also in other cellular processes such as DNA damage repair. To gain insight into the pathophysiology of RBS and ESCO2 function, we investigated the sensitivity of RBS lymphoblastoid cell lines to a diverse set of stress agents including DNA damaging agents. We found that RBS cells show increased cell death in response to mitomycin C (MMC) but not to camptothecin treatment, indicating selective sensitivity to the mechanism of DNA damage. Cell cycle analysis of RBS cells treated with a low dose of MMC (0.3 mM) showed accumulation of cells in S phase at 18 to 24h and later a G2/M arrest with subsequent apoptosis. Treatment with higher dosages (1.4 mM) resulted in S-phase arrest and induced a similar high level of apoptosis in RBS and normal cells. RBS cells were also more sensitive to G418 and sodium orthovanadate, but not to staurosporine or okadaic acid. Despite the apparent absence of cell cycle disturbance after G418 or orthovanadate treatment we found that, 24 and 48 hr after orthovanadate treatment, p53 induction was greater in RBS cells, implicating a p53 triggered cell death. These observations will help define the key defect in Roberts Syndrome.

論文審査の結果の要旨

本研究は、アザラシ肢を伴う常染色体性劣性遺伝病であるロバーツ症候群のリンパ芽球におけるさまざまなストレス薬剤に対する感受性を調べることにより、本疾患の病態の解明を目指すものである。EBV による不死化リンパ芽球を患者と正常コントロールの血液から樹立し、これに対して Mitomycin C、Orthovanadate、G418、staurosporine、Okadaic acid、Camptothecin の各薬剤を負荷したところ Mitomycin C、Orthovanadate、G418 について、高感受性を認めた。更に細胞周期、アポトーシス、p53 の発現レベルについて解析したところ、Mitomycin C はロバーツ症候群リンパ球において細胞周期の異常をきたし、Orthovanadate は p53 の発現を刺激し、アポトーシスは３剤とも亢進が認められた。著者らはすでに本疾患の原因遺伝子が sister chromatid cohesion に重要な ESCO2 であることを同定しており、本研究はその分子異常の細胞学的な解析にあたり、この成果は本疾患の病態解明に重要と考えられ、学位に値するものと認める。