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G-BANDING ANALYSIS OF CHROMOSOME ABERRATIONS IN HIROSHIMA ATOMIC BOMB SURVIVORS

G分染法による広島原爆被爆者の染色体異常の解析

TOSHIO SOFUNI, Sc.D. 祖父尼俊雄
HACHIRO SHIMBA, M.S. 榛葉八郎
KAZUO OHTAKI, B.S. 大滝一夫
AKIO A. AWA, Sc.D. 阿波章夫

放影研
RERF

RADIATION EFFECTS RESEARCH FOUNDATION
財団法人 放 射 線 影 響 研 究 所

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TOSHIO SOFUNI, Sc.D. (祖父尼俊雄); HACHIRO SHIMBA, M.S. (榛葉八郎);
KAZUO OHTAKI, B.S. (大滝一夫); AKIO A. AWA, Sc.D. (阿波章夫)

Department of Clinical Laboratories
臨床検査部

SUMMARY

A total of 896 metaphases obtained from 2-day culture of peripheral blood lymphocytes of 23 heavily exposed atomic bomb survivors of Hiroshima were examined first by an ordinary staining method, and then reexamined after trypsin-G-banding stain. There were 348 metaphases identified as having abnormal karyotypes by either one or both methods. Of these aberrant cells, 293 were found to have chromosome aberrations by the ordinary stain, and 6 of the 293 were identified as normal by G-banding stain, 4 of which were misjudged in the ordinary preparation due to the presence of partially distorted chromosomes. Furthermore, there were 55 metaphases in which abnormalities were detected only by G-banding, including paracentric inversions, and intra- and interchanges of chromosome segments with equal lengths.

INTRODUCTION

In our previous observations on the somatic chromosomes of A-bomb survivors of Hiroshima and Nagasaki, we observed that cells with radiation-induced chromosome aberrations have persisted among the circulating lymphocytes for at least three decades after exposure to A-bomb irradiation, and that the frequency of the aberrant cells is in general proportional to the radiation dose. The majority of such aberrant cells were identified as having symmetric exhanges (or stable-type aberrations consisting of reciprocal translocations and

要 約

23例の広島強度原爆被爆者の末梢リンパ球を2日間培養して得た896個の中期分裂像について，まず通常染色法で分析し，ついでトリプシンG分染法を施したのちに再分析した．両法のうち少なくとも一方の方法で染色体異常を認めた細胞は348個で，これらの中で293個は通常法により異常と判定された．通常法で異常とされたもののうち，G法で正常と判定された細胞は6個で，そのうち4個は染色体の部分的な歪みに基づく誤りであった．一方，G法によってのみ異常が識別された細胞は55個で，これらの異常は偏動原体逆位や染色体腕間および染色体間の等長交換であった．

緒 言

広島・長崎の原爆被爆者の体細胞染色体に関するこれまでの観察結果から，放射線誘発性染色体異常を示す細胞が，原爆被爆後少なくとも30年にわたり循環血液中に残存していること，また異常細胞の頻度は一般に被曝線量に比例していることが認められている．このような異常細胞の多くは相称性交換（相互転座や狭動原体逆位のような安定型異常）

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pericentric inversions), while those with asymmetric aberrations, such as dicentrics and rings (also referred to as unstable-type aberrations), were rather infrequent.[1,2] These findings prompted us to examine the radiation-induced chromosome aberrations in the somatic cells of A-bomb survivors in further detail, by comparing the results derived from a conventional staining and a trypsin-Giemsa banding method, in order to obtain more accurate information about the type and frequency of induced symmetric aberrations. The purpose of this study was also to evaluate the microscopic criteria presently in use for the detection of symmetric exhanges observed in A-bomb survivors.

MATERIALS AND METHODS

Twenty-three A-bomb survivors of Hiroshima, participants in the RERF Adult Health Study who receive biennial physical examinations,[3] were selected for study. They had had a previous cytogenetic examination in our laboratory. In each instance, more than 10% of cells from their cultured lymphocytes showed radiation-induced chromosome aberrations. Their estimated exposure doses ranged between 101 and 850 rad of mixed gamma and neutrons.[4,5] Past radiation history other than A-bomb exposure was carefully reviewed for each individual, and it was found that none of them had received either radiation therapy, including radioisotope treatment, at any time in the past or any extensive diagnostic irradiation within the year preceding blood drawing.

For all of these cases, lymphocyte cultures were successful using a whole blood culture method.[6] Culture incubation time for this study was 52 hours, during the last two of which colchicine was added. The cells were first treated with a hypotonic solution consisting of a mixture of potassium chloride and sodium citrate, then fixed by an acetic acid-methanol solution, and finally were spread and dried on slides by mild flaming. The slides thus prepared were stained for 20 minutes with 5% Giemsa solution diluted by a phosphate buffer solution at pH 6.8, and then washed and dried.

Well-spread metaphases were selected and photographed randomly under the microscope, without mounting the coverslip on the slide. The exact location of each metaphase on the slide

を有しており，一方，二動原体染色体や環状染色体（不安定型と呼ばれる）のような非相称性交換を有する細胞はかなり少ない.[1,2] このようなことから，原爆被爆者の体細胞における放射線誘発性染色体異常をさらに詳細に分析すること，すなわち通常染色法とトリプシンG分染法による結果を比較して，誘発された相称性異常の種類と頻度についてより正確な資料を得ることが重要となってきた．また，本研究により，これまでの原爆被爆者における相称性異常が顕微鏡下においてどのような基準で識別されたかについても検討を加えることができた．

材料および方法

以前に行った細胞遺伝学的調査結果に基づいて，放射線影響研究所において2年ごとに健康診断を受けている成人健康調査集団[3]から23名の広島の原爆被爆者を選択した．これらの例はいずれも培養リンパ球において10%以上の細胞に放射線誘発性の染色体異常が認められたものである．被曝推定線量はガンマ線と中性子の総和で，101 rad から 850 rad の範囲内であった.[4,5] 各人の原爆被爆以外の被曝歴について注意深く検討したところ，過去に放射線治療やラジオアイソトープ照射を受けた者や，採血前1年間にかなりの診断用放射線量を受けた者はいなかった．

全血培養法[6]によるリンパ球培養はこれら全例において成功した．本研究における培養時間は52時間で，コルヒチン処理は最後の2時間に行った．細胞を塩化カリウムとクエン酸ナトリウムの混合溶液で低張処理をし，酢酸・メタノール液で固定した後に，火焔乾燥によって標本を作製した．このようにして作製した標本をpH 6.8のリン酸緩衝液で薄めた5%ギムザ液で20分染色し，水洗後，乾燥した．

標本をカバーグラスで封入せずに，直接顕微鏡下でよく広がった中期分裂像を選び，写真撮影をした．同じ中期分裂像を再び検査するために各中期分裂像

TABLE 1 TYPE & FREQUENCY OF CHROMOSOME ABERRATIONS BY T65 DOSE (HIROSHIMA)*

表 1　T 65線量別による染色体異常の種類と頻度（広島）*

T65 Dose (rad)	Cases	Cells examined	(a) dic + r	(b) t + inv	(c) Total	(a)/(c)
1-99	70	6458	31(0.48%)	123 (1.90%)	154 (2.38%)	0.20
100-199	137	12632	64(0.51)	509 (4.03)	573 (4.54)	0.11
200-299	72	6482	49(0.76)	577 (8.90)	626 (9.66)	0.08
300-399	43	3896	41(1.05)	495(12.71)	536(13.76)	0.08
400-499	30	2866	17(0.60)	426(14.86)	443(15.46)	0.04
500+	34	3219	40(1.24)	587(18.24)	627(19.48)	0.06
Total	386	35553	242(0.68)	2717(7.64)	2959 (8.32)	0.08

*Unpublished data cited from Awa et al. 阿波ら（未発表）より引用.
dic: dicentric; r: centric ring; t: reciprocal translocation; inv: pericentric inversion.
dic：二動原体染色体，r：環状染色体，t：相互転座，inv：狭動原体逆位.

was recorded, for subsequent reexamination of the same metaphases. After routine observations, the slides were soaked with tetrachloroethylene to remove immersion oil from the slide, then in an acetic acid-methanol mixture to destain the cells, washed by tap water, and dried.

The trypsin-G-banding technique employed here was essentially the same as the original method of Seabright:[7] the slides were treated for 1 to 15 seconds in 0.2% trypsin solution (1:250, Difco) dissolved in a calcium-, magnesium-free balanced salt solution, then washed in running water, and restained with a 5% Giemsa solution at pH 6.8.

The banded metaphases, which had been previously photographed, were relocated, reexamined, and again photographed. Karyotype analyses from the printed photographs of the same metaphases derived from the two different staining methods were made separately, so that the types and frequencies of radiation-induced chromosome aberrations were evaluated independently (the ordinary staining method is abbreviated hereafter as O-method, and G-banding as G-method).

RESULTS

Table 1 summarizes our previous cytogenetic findings among 386 Hiroshima A-bomb survivors (Awa et al, cited from unpublished data), and the increase in the frequency of exchange aberrations with increasing doses is clear. Symmetric aberrations, such as reciprocal translocations and

の正確な位置を記録した．通常の顕微鏡観察の後に，標本上のイメルジョン・オイルをテトラクロルエチレンで洗い落し，酢酸・メタノール液で脱色し，水道水で洗った後に乾燥した．

本研究に用いたトリプシンG分染法は基本的にはSeabright[7]の原法と同じである：標本をカルシュウム・マグネシウムを含まない等張塩類溶液に溶解した0.2%トリプシン液（1：250, Difco）で1－15秒処理をし，水道水で洗い，pH 6.8の5%ギムザ液で再染色した．

最初に写真撮影した中期分裂像を顕微鏡上に選び，分染された中期分裂像を再び写真撮影した．同一の中期分裂像についての二つの異なる染色法で得られた写真を基に，それぞれ別々に核型分析を行い，放射線誘発性染色体異常の種類と頻度を分析した．（通常の染色法をO法，G分染法をG法と以下に呼ぶこととする．）

結　果

386例の広島原爆被爆者の細胞遺伝学的研究の結果については表1に要約したように，交換型染色体異常の頻度は線量の増加と共に増えていることが明らかである（阿波ら，未発表資料より）．相互転座や狭動原体逆位のような相称性異常は非相称性異常より

pericentric inversions, were found to predominate over asymmetric ones, and they were the major components contributing to the dose-aberration relationship. In contrast to the predominance of symmetric aberrations, asymmetric exchanges (dicentrics and rings) were far less frequent, being less than 10% of the total number of aberrant cells analyzed, although a dose-response relation was nonetheless observed.

The results of karyotype analysis of 896 metaphases (using both O-method and G-method) derived from 23 Hiroshima survivors are shown in Tables 2 and 3. Though the number of metaphases as well as the number of cases was small, there was in general a dose-dependent increase in the frequency of cells with chromosome aberrations for either method. The frequency of aberrant cells was clearly higher in the G-method than in the O-method series for every dose group (Table 2). The ratio of aberrant cells by the O-method to that by the G-method was 0.86 (293 for O vs 342 for G).

Of the 896 metaphases analyzable by both methods, 548 cells were identified as normal, while aberrations were observed by either one or both methods in the remaining 348 cells (Table 3). The frequency of asymmetric aberrations (dicentrics, rings, acentric rings, and acentrics with terminal and interstitial deletions) was extremely low, there being only 17 in 348 cells (or 5% of the total number of aberrant cells).

Further comparison of the karyotypes from the 348 cells with aberrations showed that the aberrations were identical in 151 cells (43%), while they were not identical in the remaining 197 cells. The latter group of aberrant cells was further divided into three subgroups as shown in Table 3; (a) though abnormalities were identified by both methods, the types of aberrations were not identical* (136 cells), (b) abnormalities were detected only by G-method but not by O-method (55 cells), and (c) G-method failed to detect any abnormality in cells which had already been identified as abnormal by O-method (6 cells). For these six cells there were four in which one chromosome by O-method appeared to be abnormal, since it was either overcontracted

もはるかに多くみられ，これが線量と異常頻度との相関関係の重要な要因となっている．相称性異常が大多数を占めているのに対し，非相称性異常（二動原体染色体と環状染色体）はかなり少なく，総異常細胞の10%にも満たない．しかしながら線量反応関係は認められている．

23例の広島の被爆者から得られた896個の中期分裂像に対するOとGの両法による核型分析の結果を表2と3に示した．例数および細胞数は少ないが，いずれの方法においても染色体異常をもつ細胞の頻度は一般に線量と共に増加している．異常細胞の頻度はいずれの線量群においてもO法よりもG法の方が明らかに高い（表2）．G法に対するO法の異常細胞の比率は0.86（O法293個に対しG法342個）であった．

両法で分析できた896個の中期分裂像のうち548個は正常と判定され，残りの348個には両法ないしはいずれかの方法によって異常が認められた（表3）．非相称性異常（二動原体染色体，環状染色体，無動原体環状染色体と端部および中間部欠失を伴う染色体断片）の頻度は非常に低く，348細胞中わずか17個（総異常細胞の5%）であった．

348個の異常細胞の核型についてさらに比較すると151個（43%）において異常が一致しており，残りの197個においては異常の種類が一致しなかった．一致しなかった異常細胞を表3に示したように3群に分類した：（a）両方によって異常が認められたが異常の種類が異なるもの*（136細胞），（b）O法では識別できずG法によってのみ異常が識別できたもの（55細胞），（c）O法で異常と判定したがG法では正常であったもの（6細胞）．O法でのみ異常と判定された6細胞のうち4細胞では染色体が収縮したりねじれた

* *For example, a pericentric inversion by O-method was in fact determined to be a reciprocal translocation or even more complicated exchange by G-method.*

例えばO法によって狭動原体逆位とされたものが実際はG法によって相互転座，あるいは，さらに複雑な交換型異常であると判定された．

TABLE 2 FREQUENCY OF ABERRANT CELLS BY T65 DOSE IN 23 HEAVILY EXPOSED A-BOMB SURVIVORS OF HIROSHIMA

表 2　23例の広島強度原爆被爆者のT 65線量別による異常細胞の頻度

T65 Dose (rad)	(mean)	Cases	Cells analyzed	Aberrant cells	
				O-method	G-method
100-199	(157.8)	8	295	68(23.1%)	78(26.4%)
200-299	(258.2)	6	257	80(31.1)	92(35.8)
300-399	(331.7)	3	137	52(38.0)	63(46.0)
400-499	(405.7)	3	85	25(29.4)	29(34.1)
500+	(710.7)	3	122	68(55.7)	80(65.6)
Exposed total	(311.1)	23	896	293(32.7)	342(38.2)
Total cells photographed				1758	1114

TABLE 3 COMPARISON OF KARYOTYPES OF THE SAME METAPHASES BETWEEN ORDINARY & G-BANDING METHODS

表 3　通常法とG分染法による同一中期分裂像の核型の比較

O-method		G-method	Karyotype	Karyotypes
N	:	N	identical	548
AB	:	AB	identical	151
AB	:	AB	nonidentical	136
N	:	AB	nonidentical	55
AB	:	N	nonidentical	6
Total				896
Nonidentical karyotypes				197

N: normal karyotype, AB: abnormal karyotype.

N：正常核型，AB：異常核型.

or unusually twisted, but by G-method its banding pattern proved to be normal. In the remaining two cells, an exchange could conceivably have occurred at the negative regions of the distal part of certain chromosomes, indicating that even the banding technique may fail to detect exchanges of this type.

To further simplify the results observed from the G-method, the 348 cells with chromosome aberrations were classified into three groups according to the types of aberrations by scoring the number of breaks involved in each exchange; i.e., S-type cells as having simple aberrations caused by one or two breaks, C-type as complex interchanges with three or more breaks involved, and U-type in which the types of exchanges could not be determined. Table 4 shows that there were 31 U-type cells in which aberrations

ために異常と判定されたもので，G法でみると分染パターンは正常であった．残りの2個の細胞では染色体端部の染まらないバンドの間に異常が生じており，そのために分染法を用いてもこの種の異常を識別することはできないものと思われる．

G法によって得られた結果についてさらに検討を加えるために，染色体異常をもつ348細胞を異常に関与する切断数に従って三つの群に分類した：一つないし二つの切断によって生じた単純な異常をもつものをS型細胞，三つないしはそれ以上の切断によって生じた複雑な異常をもつものをC型細胞，異常の種類を決定できなかったものをU型細胞とした．表4に示したように，31個のU型細胞では異常が非常に

TABLE 4 CLASSIFICATION OF ABERRANT CELLS DETECTED BY ORDINARY & G-BANDING METHODS

表4　通常法およびG分染法によって識別された異常細胞の分類

O-method • G-method	Metaphases			Total
	S	C	U	
AB : AB	225	35	27	287
N : AB	50	1	4	55
AB : N	6	-	-	6
Total	281	36	31	348

N: normal karyotype, AB: abnormal karyotype.
S: simple-type with 1 or 2 breaks, C: complex-type with 3 or more breaks, U: unidentifiable-type (see text)
N：正常核型，AB：異常核型.
S：一つあるいは二つの切断による単純型，C：三つあるいはそれ以上の切断による複雑型，U：異常識別不能型(詳細は本文参照).

were so complicated that even the banding method could not specify the types of exchanges. There were 36 C-type cells which showed various types of complex exchanges. About 20% of the 348 cells, or 67 cells, belongs to either C- or U-type, indicating that the aberrant cells persisting to date carry really complicated structural rearrangements of chromosomes.

Although the types of exchanges detected by the G-method but not by the O-method among the present cases have not been fully characterized yet, preliminary analysis showed that these aberrations consisted of paracentric inversions (in 12 chromosomes so far confirmed), intra- and interchanges of chromosome parts with equal lengths, or even with unequal lengths that were judged nonetheless to be within the normal limits of variation by O-method. As for complex exchanges, insertions of chromosome segments of both inversion- and translocation-types, and sequential translocations involving more than three chromosomes were frequently noted. These exchanges undoubtedly contributed to an elevated frequency of aberrant cells in the G-method series.

In the O-method series, aberrations designated as deletions were frequently observed, in which only one of the chromosomes in the complement was shorter than the partner, but about a half of them were classified as reciprocal translocations by the G-method because the corresponding abnormal counterpart could be identified.

複雑なために分染法を用いても異常の種類を決定できなかった．36個のC型細胞ではいろいろな種類の複雑な異常がみられた．348細胞の約20%，つまり67個の細胞がCあるいはU型細胞であり，このことは今日まで残存している異常細胞が非常に複雑な染色体の構造異常を有していることを示している．

これまでのところ，O法では認められず，G法で見い出された異常の種類についてはまだ十分に解析されていないが，予備的解析によればこれらの異常の中には偏動原体逆位(これまで12本の染色体に確認されている)，染色体腕間あるいは染色体間の等長交換，あるいは等長交換ではなくともO法では正常範囲内とみなされる異常が含まれていた．複雑な異常としては逆位型や転座型の染色体部位の挿入や，3本あるいはそれ以上の染色体が関与している連続型転座がかなり認められた．これらの異常がG法における異常細胞の出現頻度を増加させていると思われる．

O法で欠失と判定された異常，つまり染色体の1本が相同対よりも短かいもののうち，約半分はG法によって相互転座と判定された，すなわち転座に関与するもう一方の異常染色体をG法で識別することができた．

DISCUSSION

The majority of exchange aberrations observed in the peripheral blood lymphocytes of Hiroshima and Nagasaki A-bomb survivors are the symmetric type, while cells with asymmetric aberrations are very low in frequency.[1,2] It seems likely that in these individuals irradiated more than 20 years ago, over this period of time the cells with asymmetric exchanges would have been eliminated from the lymphocyte population due perhaps to mitotic disturbances. In this connection, the symmetric exchange, therefore, would seem to be a useful indicator for evaluating the dose-aberration relationship, especially in those exposed to large doses of radiation long before cytogenetic examination.

Unfortunately, microscopic detection of symmetric exchanges is limited in the O-method, since the criteria for the identification of individual chromosomes are based only on relative lengths and arm ratios (or indices) including a certain amount of variability for each chromosome. A large fraction of such aberrations is thus likely to be overlooked.[8,9] For example, Sasaki[9] reported from his in vitro irradiation experiments of human lymphocytes that the frequency of symmetric aberrations was only about 20% of asymmetric exchanges. Since the probability of both symmetric and asymmetric aberrations being induced by irradiation is assumed to be equal, about 80% of the symmetric exchanges remain undetected by the O-method.

By the use of banding techniques, it was possible to analyze the same metaphases in two different ways: first by the O-method, and then by the G-method, so that the frequencies of chromosome aberrations derived from these two methods can be compared directly with each other. The present results showed that the frequency of aberrant cells was definitely higher in the G-method series than in the O-method series. The O-method detected 86% of the aberrant cells identified by the G-method, which was higher than previously assumed.[8-10]

Some types of exchanges were observed by the G-method that could not be identified by the O-method. Paracentric inversion is a typical example, the frequency of which was close to that of the total number of asymmetric aberrations observed in the sample. In addition to paracentric inversions, several types of insertions and interchanges of chromosome

考　察

広島・長崎原爆被爆者の末梢リンパ球にみられる交換型異常の多くは相称性交換であり，他方，非相称性交換の出現頻度は非常に低い.[1,2] 被爆後20年以上経過したこれらの人々では，この間に非相称性交換をもつ細胞が分裂上の障害のためにリンパ球集団から淘汰されたものと考えられる．このことは相称性交換が特に高線量被曝後長年月を経たような例では，線量と異常の相関関係を検討する上で重要な指標となることを示唆している．

しかしながら，これまでの通常の染色法では染色体の相対的長さや腕比（着糸点指数）のみで個々の染色体の識別をし，しかも個々の染色体にある程度のばらつきのみられることから，顕微鏡下で相称性交換を識別することには限度があった．そのためこの種の異常の多くは見落されていると考えられている.[8,9] たとえば Sasaki[9] のヒトリンパ球の試験管内照射実験では，相称性異常の頻度は非相称性異常のおよそ20%にすぎないと報告されている．放射線によって誘発される相称性と非相称性異常の確率は等しいと想定されることから，相称性異常の約80%は通常の染色法によって識別できないものと考えられる．

分染法の応用によって同じ中期分裂像を二つの異なる方法を用いて分析することができるようになった：最初に通常の染色法を用い，その後でトリプシンG分染法で分析し，二つの方法で得られた染色体異常の頻度を互いに直接比較することができる．本研究の結果によると，異常細胞の頻度はO法に比べてG法の方が明らかに高かった．O法で識別できた異常細胞はG法の86%であり，これはこれまで推定されたものよりはるかに高い頻度である.[8-10]

G法で識別できた交換型異常の中にはO法で識別できないものがあった．その典型的な例は偏動原体逆位で，その出現頻度は非相称性異常の頻度にほぼ一致していた．偏動原体逆位の他に，各種の挿入，あるいは染色体間の等長や非等長の交換もかなり

segments with both equal and unequal lengths were also detected frequently.

Another noteworthy feature was that some of the translocations, which were identified as simple reciprocal types by the O-method, were in fact more complicated involving more than three breaks, and often showed sequential interchanges. Further detailed analyses by the G-method are in progress in terms of the types of aberrations, number of breaks per cell, and the sites of break-points in the somatic cells of A-bomb survivors.

みられた.

さらに注目すべきこととしては，O法で単純な相互転座と判定されたものの中には実際にはさらに複雑で，3本あるいはそれ以上の染色体が関与し，しかも連続的に交換が生じているものがしばしばみられた．原爆被爆者の体細胞における染色体異常の種類，細胞当たりの切断数および切断部位については目下G法を用いて詳細に分析中である．

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