Chapter 5

In vitro validation of translocation frequency obtained by FISH with G-banding
5.1. Introduction

G-banding was one of the earliest techniques that enabled detailed visualization of structural aberrations due to radiation (1-2). Although G-banding enables detailed analysis of the whole karyotype, studies performed in the 1970’s concluded that, it cannot be recommended for cytogenetic routine analyses in medical radioprotection monitoring, without suitable automated scoring techniques (3). In the 1980’s, with the introduction of FISH, radiation biodosimetry experienced a breakthrough in the analysis of structural chromosome aberrations with emphasis on translocations (TL). Despite this, with the help of automated metaphase finders, many laboratories, still undertake G-banding to observe chromosome aberrations due to radiation (4-6).

TL measurement with FISH employs DNA probes specific only for a subset of the genome, e.g., painting chromosomes 1 and 3 (15% of the genome) resulting in the detection of 27% of all TL (7). The total genomic TL frequencies are extrapolated from the DNA content of painted chromosomes. Assuming that radiation results in a random distribution of chromosome breaks, such scaling can be performed accurately. Thus, full genomic TL frequencies are accurately obtained after selectively painting only a small fraction of the genome. This important finding was determined by comparing reciprocal TL frequencies in the same individuals as measured by FISH and G-banding (7). However subsequent studies reported under or over representation of painted chromosomes to form radiation induced TL. This raised a concern over the suitability of dose estimation in analyzing TL by selected painting of few chromosomes.

This called for validation of results obtained by FISH with conventional techniques like G-banding. Many investigations have been undertaken previously to verify whether TL frequency detected by FISH is indeed the same as that observed by the classical G-banding, which enables detection of aberrations in all chromosomes (6, 7, 8, 9). Studies have also been undertaken since the proposition of Lucas’ hypothesis, to determine whether radiation induced chromosomal aberrations are random or non-random (10-17). Parameters like choice of chromosome(s) selected for FISH (11), scoring nomenclature employed and reports of higher TL frequencies as compared to
DC have questioned the use of this technique for biodosimetry. Hence it was of interest to check these parameters and the usefulness of TL for biodosimetry.

In this regard, the present study was undertaken to validate FISH based TL yields with those obtained by G-banding and from the information gleaned by G-banding to ascertain the nature of radiation induced chromosomal aberrations in being random or non-random.

### 5.2. Methodology

Irradiated cultured peripheral blood lymphocytes used to construct dose response curves (detailed in Sections 3) were used in this study. Only 5 doses were considered for validation, apart from the sham-irradiated control – 0.25Gy, 0.5Gy, 1Gy, 2Gy and 4Gy. Metaphases obtained from these lymphocytes were subjected to FISH and G-banding as given in Sections 2.3.1.5 and 2.3.1.7.

For FISH, 250-1000 metaphases were scored for TL depending on the dose (18, 19). These were further simplified using the PAINT nomenclature (20, 21). For G-banding analysis, 25-200 metaphases were analysed and karyotyped according to the radiation dose. The TL yields obtained at different radiation doses by G-banding were tabulated and compared with those obtained by FISH. The chromosomal involvement of each chromosome in TL formation as ascertained by G-banding was noted. This data was normalized and plotted against chromosome DNA content as given by Morton (22). Statistical analysis (‘t’ test) was performed using the online statistical software - Instat Graphpad when necessary.

### 5.3. Results

It was observed that as the radiation doses were raised, the abnormal metaphases and frequency of chromosomal alterations increased as well. The majority of chromosomal alterations were of the structural type, of which the ones considered in this study were TL.
The data obtained for TL by G-banding was tabulated (Table 5.1). A dose-dependent increase in the yield of TL was observed. A comparison of TL frequencies by FISH and G-banding is depicted in Figure 5.1. TL frequencies obtained by FISH and G-banding matched well ($r^2 = 0.9994$). The ‘t’ test also gave a $p$ value of 0.226 confirming the same. Normalised values for TL yields scored by G-banding plotted against chromosome size are shown in Figure 5.2. Chromosome 1 was more frequently involved in structural chromosomal alterations, followed by chromosomes 7, 10, 8 and 4. Some complex aberrations observed by G-banding are shown in Figure 5.3.

### Table 5.1

**Translocations scored by G-banding in peripheral blood lymphocytes exposed in vitro to different doses of 0.74 Gy/min $^{60}$Co $\gamma$ radiation.**

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>CS</th>
<th>TL</th>
<th>TL / Cell ± SE</th>
<th>TL Distribution</th>
<th>$u$</th>
<th>$\sigma^2/y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>200</td>
<td>1</td>
<td>0.005 ± 0.005</td>
<td>199</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>0.25</td>
<td>200</td>
<td>4</td>
<td>0.020 ± 0.010</td>
<td>196</td>
<td>-1</td>
<td>-0.17</td>
</tr>
<tr>
<td>0.50</td>
<td>220</td>
<td>8</td>
<td>0.036 ± 0.013</td>
<td>214</td>
<td>5</td>
<td>8.07</td>
</tr>
<tr>
<td>1.00</td>
<td>59</td>
<td>4</td>
<td>0.068 ± 0.034</td>
<td>55</td>
<td>4</td>
<td>-0.27</td>
</tr>
<tr>
<td>2.00</td>
<td>70</td>
<td>10</td>
<td>0.143 ± 0.045</td>
<td>60</td>
<td>10</td>
<td>-0.81</td>
</tr>
<tr>
<td>4.00</td>
<td>25</td>
<td>19</td>
<td>0.760 ± 0.174</td>
<td>9</td>
<td>14</td>
<td>-1.11</td>
</tr>
</tbody>
</table>

CS – Cells scored  TL – Translocation  SE – Standard Error

$u$ – Dispersion coefficient  $\sigma^2/y$ – Relative Variance
Figure 5.1

Comparison of translocations obtained by FISH (WCP 1 & 3) and G-band in peripheral blood lymphocytes exposed \textit{in vitro} to different doses of Co-60 gamma radiation.

\[ r^2 = 0.9994 \]
Figure 5.2

Translocation yields (normalised) obtained by G-banding in peripheral blood lymphocytes exposed *in vitro* to different doses of Co-60 radiation and plotted against DNA content of each chromosome.
Validation of in vitro translocation frequency obtained by FISH with conventional G-banding

Figure 5.3: Chromosomal aberrations observed by G-banding in peripheral blood lymphocytes exposed \textit{in vitro} to Co-60 gamma rays.

Translocation involving chromosomes 1 and 12

Complex aberrations induced at 4Gy
5.4. Discussion

TL analysis using G-banding, allows the identification of rearrangements involving any chromosome in the genome and also the precise location of breakpoints within each chromosome. But, it is more time consuming, tedious and may not always be feasible for biodosimetry due to the fact that many thousands of cells need to be scored at low doses. The advent of FISH (23) has revolutionized the field of radiation biodosimetry by making the analysis of TL simpler and faster than that possible by conventional methods (7).

During the assessment of TL analysis for biodosimetry, it was soon clear that several factors ought to be studied more carefully. A number of biological factors have been proposed to modulate the induction, chromosomal distribution or persistence of radiation-induced TL, including size (24-28), chromatin organisation (29-34), nuclear architecture (35), differential DNA repair or gene density (36-37), heterogeneous telomere shortening (39), and higher sensitivity of interstitial telomeric sequences (40-42).

Numerous in vitro studies have been conducted in the past validating TL yields detected by FISH and G-banding and showing them to be non-variable (7-8, 42). Our study also shows a similar trend. TL yields obtained in vitro by G-banding and FISH show good correlation (r² = 0.9994). The normalized values for TL yields involving chromosomes 1 and 3 by FISH show an over involvement of chromosome 1 with respect to chromosome 3 (Figure 3.4). The results show that the frequencies of TL in individual chromosomes as well as between specific chromosomes of the selected group are usually linearly proportional to the DNA content of chromosomes as proposed by Lucas et al (7). Similar tendency as FISH, of chromosome 1 being involved in most aberrations was observed even with G-banding. Chromosome 1 seems to be over involved with 17% of all TL. Chromosome 7 was next with 9%. Sommer et al (43) also provide report of under and over representation of certain chromosomes compared to the expected values. In fact, chromosomal involvement of TL based on DNA content was seen in the descending order with larger chromosomes.
being more involved when compared to smaller chromosomes with minor variation (7). Our TL data obtained for G-banding also shows a DNA proportional involvement of most chromosomes with few exceptions.

It can be hypothesized that an unequal distribution of aberrations on individual chromosomes can be attributed to an uneven distribution of either repair genes or genes regulating the cell cycle on the chromosomes and that these genes can be specifically activated or deactivated by exposure agents like radiation or their metabolites. The results of Surralles et al (38) indicated that the level of excision repair synthesis is higher in high gene density chromosomes (chromosome 1 in our case) and, therefore, these chromosomes are preferentially repaired. Moreover, transcription-coupled repair, a specialized nucleotide excision repair pathway, was shown to be organized in clusters incorporating predominantly early-replicating gene-rich bands within the human genome. The results of Puerto et al (16) further stress on the fact that chromosomes with high gene density are preferentially repaired in human cells leading to greater involvement in aberration formation. In our study we found chromosome 1 to be involved in a number of radiation-induced breakpoints. Since these breakpoints are found to be associated with several malignancies particularly hematological and as TL play a crucial role in tumor development these preliminary findings may provide some clues for the early detection of malignancies.

The use of FISH to detect TL is more sensitive than conventional techniques because of the accuracy and ease of detection as well as the large number of cells that can be analyzed. Though there may be inter-individual variation, chromosome breakage depends mainly on the length of the chromosome. Universally accepted as an accurate method of detecting TL, G-banding is much too labor intensive for exposure and risk-assessment applications. The FISH method is much faster and demonstrates identical results when scaled to full genome, providing a practical biomarker for applications that requires the scoring of large numbers of cells and individuals (44).
5.5. Conclusion

The results from our study support existing literature in that FISH based TL yields agree well with those obtained by G-banding. While the conventional cytogenetic analysis is the method of choice for the determination of unstable type of aberrations, the FISH technique is a rapid, sensitive, and reliable method for the detection of both stable and unstable structural rearrangements. This method is suitable for analyzing even low-dose radiation exposures and has been used in several studies for both retrospective dosimetry and biological dosimetry. In comparison, FISH appears to be more sensitive than the conventional technique in the detection of genomic frequency of TL induced by irradiation.

FISH has continued to evolve with time, giving rise to techniques like multicolour FISH (mFISH), which adds more colour and gives more precise experimental data as TL can be observed in all chromosomes. Hence, the next section deals with comparison of FISH based TL data with that obtained by mFISH.

5.6. References


