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Technical Note

Interphase Quantitative Fluorescence *in Situ* Hybridization (IQ-FISH)

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Abstract

Fluorescence in situ hybridization (FISH) seems to be the most applicable and versatile molecular cytogenetic platform for visual interphase chromosome analysis offering a variety of opportunities for studying chromosomal structure and behaviour at the highest microscopic resolution and at all stages of the cell cycle. Quantitative assessment of FISH results has been repeatedly shown to increase the efficiency of FISH-based approaches. Here, we explore the potential of interphase quantitative FISH (IQ-FISH), which is the result of combining interphase FISH with an approach to FISH signal quantification. Firstly, IQ-FISH is applicable for high-resolution molecular cytogenetic analysis of somatic genome variations in large populations of interphase cells (n>1000) inasmuch as it allows for the differentiation between genomic variations (chromosome abnormalities) and specific nuclear chromosomal organization. Secondly, IQ-FISH may be a basis for more sophisticated interphase molecular cytogenetic techniques (i.e. multicolour banding of interphase chromosomes). Thirdly, IQ-FISH depicts nuclear organization by demonstrating the distribution of specific chromosomal DNA within nuclear area. Finally, IQ-FISH is a unique technique allowing quantitative

characterization of chromosomal heteromorphisms in the interphase nuclei. It is to note that interphase chromosome heteromorphisms are rarely evaluated because of technical limitations. In conclusion, IQ-FISH appears to be a handy technique to increase the efficiency of interphase chromosome analysis.

Keywords
Interphase chromosomes; fluorescence *in situ* hybridization; quantification

1. Introduction

Since the arrangement of interphase chromosomes represents the driving force of genome behaviour at the highest level of the organization in the nucleus, it is a major focus of current biomedical research. Moreover, single-cell analysis of intercellular genome variability benefits from addressing numbers and structure of interphase chromosomes. To study human interphase chromosomes, one can address a variety of molecular cytogenetic techniques, which are usually based on fluorescence *in situ* hybridization (FISH) [1]. Quantitative assessment of FISH results has long been regarded as a way to increase the efficiency of molecular cytogenetic analysis by uncovering DNA content at the targeted chromosomal (genomic) loci [2, 3]. Quantitative FISH (QFISH) has been used for visualization of chromosomal loci in interphase [4-6]. Additionally, QFISH has been shown to be an integral part of analyzing chromosome variation in single cells [7-9]. Furthermore, automated and on-chip FISH-based protocols include signal quantification [10-11]. Similarly, high-resolution FISH-based techniques for multicolor painting of interphase chromosomes involve signal quantification [12, 13]. Finally, FISH-signal intensity profiles are able to depict genome architecture within human interphase nuclei [14]. Interestingly, QFISH has been demonstrated to be valuable for molecular cytogenetic diagnosis [4, 15-17]. Nevertheless, QFISH protocols are rarely used for basic and diagnostic research of human interphase chromosomes. Thus, one can suggest that there is a need for an evaluation of QFISH potential for interphase cytogenetics.

Here, we describe a combination of original interphase FISH protocols [1, 7, 9, 12] with a QFISH approach [3, 4, 14]. The combination has been termed interphase QFISH (IQ-FISH). To explore IQ-FISH potential, we have analyzed molecular cytogenetic data on chromosomal DNA variation and the arrangement in interphase nuclei of post-mitotic cells of the human brain.

2. Method

Suspension preparation of 66 fresh-frozen brain samples obtained post-mortem from individuals with schizophrenia (n=22), Alzheimer's disease (n=10) and from controls (n=34) was carried out according to a step-by-step protocol described in details elsewhere [18, 19]. FISH was performed as described in a series of our previous molecular (neuro)cytogenetic reports [19-22]. We used DNA probes (chromosome-enumeration probes) for chromosome 1 (D1Z1), 9 (D9Z1), 15 (D15Z1), 16 (D16Z3), 18 (D18Z1), and X (DXZ1). Fifty nuclei were analysed per probe for each sample (about 20000 nuclei). Quantification of FISH signals in interphase nuclei (IQ-FISH) was performed using ImageJ software developed by the US National Institute of Health

(https://imagej.nih.gov/ij/) [23] as described earlier [3]. Briefly, FISH images were acquired by a charge-coupled-device camera using separate filter set specific for each fluorochrome. Each file of acquired images was saved in black and white (8-bit) and was loaded into the ImageJ software. FISH signal areas were selected using "rectangular" selection tool to generate the plot of image containing the graph depicting the intensity profiles. The area below the graph corresponding to FISH signals was measured. The numerical values obtained from the same image were compared. Examples of IQ-FISH are shown in Figure 1.

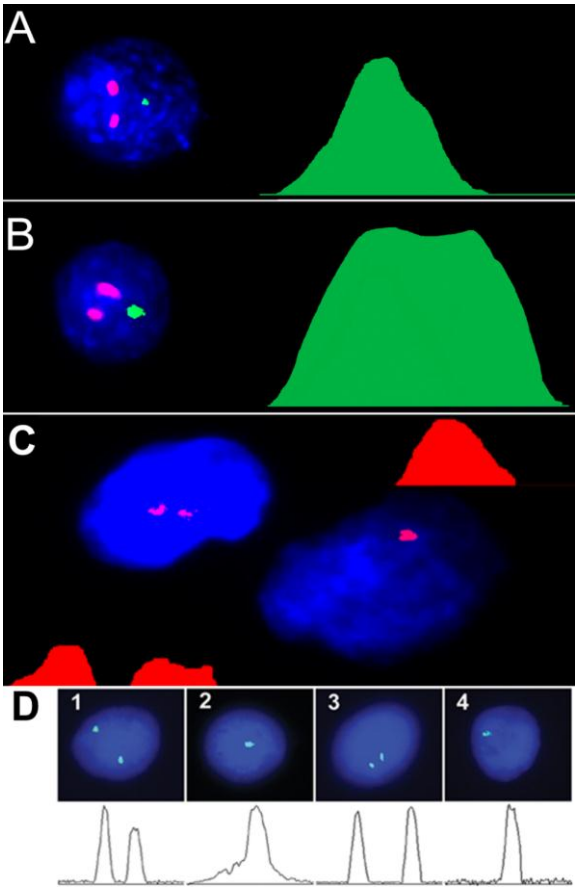

Figure 1 (A) IQ-FISH with chromosome 1-enumeration (two red signals) and chromosome X-enumeration (one green signal; relative intensity: 2120 pixels) DNA probes demonstrating true monosomy of chromosome X; (B) IQ-FISH with the same DNA probes (chromosome 1 — two red signals; chromosome X — one green signal; relative intensity: 4800 pixels) demonstrating an association of two signals, but not X chromosome loss (Copyright © Yurov et al. 2014 [28]; licensee BioMed Central Ltd). This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0); (C) IQ-FISH showing an association of chromosome 9 signals, but not monosomy; relative intensities: 2134 and 1633 pixels (left nucleus) and 3912 pixels (right nucleus) (Copyright © Vorsanova et al. 2010 [7]; licensee BioMed Central Ltd). This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0); (D) IQ-FISH showing a nucleus with two signals for chromosomes 18 (relative intensities: 2058 and 1772 pixels), (2) a nucleus

with one associated signal for chromosome 18 (relative intensity: 4012 pixels), (3) a nucleus with two signals for chromosomes 15 (relative intensities: 1562 and 1622 pixels), (4) a nucleus with one signal (monosomy) of chromosome 15 (relative intensity: 1678 pixels) (Copyright © Yurov et al. 2007 [20], an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0)).

The efficiency of IQ-FISH for scoring chromosomal loss was measured using our previous data on interphase molecular cytogenetic analysis of the same brain samples performed using interphase chromosome-specific multicolour banding or ICS-MCB (for the whole datasets, see [24, 25]). Chromosome losses were observed in 3.9% and 3.7% of neural cells when evaluated by IQ-FISH [21, 22] and ICS-MCB [12, 24, 25], respectively: no statistically significant difference ($p=0.007$) was found. Alternatively, IQ-FISH studies have shown specific intranuclear organization of pericentromeric chromosomal regions in the brain, which has been highlighted previously [12, 26]. Thus, pericentromeric DNA associations were found in 43.3% of brain cells for chromosome 1, in 21.4% of brain cells for chromosome 9, in 29.3% of brain cells for chromosome 15, in 34.8% of brain cells for chromosome 16, in 15.4% of brain cells for chromosome 18, and in 4.1% of brain cells for chromosome X. It is to mention that these patterns of nuclear chromosomal organization are highly difficult to identify without IQ-FISH analysis. Additionally, we observed chromosomal heteromorphisms in a number of samples. A heteromorphism was defined when signal intensity ratios were higher than 1:3. An increase in the length of a pericentromeric chromosomal region (qh+) was determined when signal intensities were found to be less than 1500 pixels, whereas a decrease in the length of a pericentromeric chromosomal (qh-) region was determined when signal intensities were found to be more than 4000 pixels. We found 1qh+ in 2 samples, 9qh+ in 2 samples, 16qh+ in 1 sample, 1qh- in 1 sample, and 9qh- in 2 samples. In this context, it is important to bear in mind that IQ-FISH offers a unique possibility to demonstrate accurately the presence chromosomal heteromorphisms in interphase nuclei. As we noted previously [14], QFISH provides for monitoring denature time and duration of hybridization to identify optimal hybridization conditions. It was established that 100% efficiency of FISH (i.e. the point when relative intensities ceased to change) is achieved after 960 minutes (16 hours). Here, this opportunity of FISH signal quantification has been confirmed using IQ-FISH.

3. Applications

Analyses of IQ-FISH data demonstrate the applicability of this molecular cytogenetic technique for studies of somatic genome variations. These are repeatedly described as significant contributors to human interindividual/intercellular genomic diversity in health and disease [26-28]. Our studies of the normal and diseased human brain [20-22, 26-28] have confirmed high applicability of QFISH in uncovering somatic chromosomal mosaicism in large cell populations. Interestingly, it has been reported that a QFISH protocol can be used for automated identification of gene amplifications [29]. Therefore, IQ-FISH is not limited to detection of aneuploidy/polyploidy, but is also applicable for detecting structural aberrations of interphase chromosomes.

Quantitative analysis of FISH signals is a digital basis for ICS-MCB [12, 30], a technique extending multicolour chromosomal banding to interphase cytogenetic analysis (for more details, see [9, 31]). Although the results of IQ-FISH are comparable with ICS-MCB, it is to keep in mind

that the latter is the only technique for microscopic analysis of whole interphase chromosomes at molecular resolutions and at any stage of the cell cycle [1, 9, 12, 30, 31]. One can suggest that using both IQ-FISH and ICS-MCB is likely to be the most efficient way of performing molecular cytogenetic microscopic studies of interphase chromosomes.

It has been systematically shown that ICS-MCB can uncover chromosomal organization in the nucleus [1, 9, 12, 13, 30]. Additionally, IQ-FISH using DNA probes for repetitive genomic elements highlights the intranuclear arrangements of chromosomal DNAs (i.e. distribution of satellite DNA within a nuclear area) [14]. More importantly, IQ-FISH seems to be the way of uncovering chromosomal associations in almost any FISH study of interphase nuclei.

QFISH has been previously used for the identification of parental origins of homologous chromosomes using either chromosomal heteromorphisms or structural variations (i.e. copy number variations or CNV) [32-34]. Here, IQ-FISH is shown applicable for the same purposes. Since interphase chromosomal heteromorphisms are rarely evaluated due to technical limitations, IQ-FISH may be a handy technique for the identification, especially when metaphase karyotyping is not possible.

4. Conclusions

Exploring the potential of IQ-FISH, we have found it to be a handy technique for increasing the efficiency of interphase chromosome analysis. The technique has at least four major areas of the application: (i) molecular cytogenetics of somatic genome variations (analysis of chromosomal variations in large populations of interphase cells); (ii) technological developments in interphase molecular cytogenetics (i.e. developing such sophisticated techniques as ICS-MCB); (iii) analysis of nuclear organization of interphase chromosomes (chromosomal associations or distribution of satellite DNA within nuclear areas); (iv) uncovering interphase chromosome heteromorphisms. Finally, IQ-FISH seems to be a technique allowing the analysis of phenomena, which are still impossible to address by post-genomic technologies.

Author Contributions

IYI, IVS, YBY and SGV conceived the idea and approved the technique. IYI wrote the manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

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