Chapter 7
Twenty-First Century FISH: Focus on Interphase Chromosomes

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Abstract Interphase molecular cytogenetics provides opportunities for analysis of chromosomes in almost all types of human cells at any stage of the cell cycle. Generally, interphase fluorescence in situ hybridization (I-FISH) is a basic technological platform for visualization of individual chromosomes (chromosomal regions) in single cells. The achievements of studying human interphase chromosomes have allowed numerous discoveries in chromosome research (molecular cytogenetics) and genomics (cytogenomics). In the postgenomic era, interphase chromosome analysis by I-FISH remains an important part of biomedical research. Here, we describe the spectrum of FISH applications with special emphasis on interphase chromosome biology and molecular cytogenetic/cytogenomic diagnosis.

Introduction

Fluorescence in situ hybridization (FISH) is recognized as one of essential technological platforms for molecular cytogenetics. During the last decades, FISH has been found useful for a wide spectrum of applications from molecular diagnosis to basic chromosome biology (van der Ploeg 2000; Vorsanova et al. 2010c; Yurov et al. 2013; Liehr 2017; Hu et al. 2020). Previous edition of this book contained a chapter

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dedicated to technological solutions in interphase chromosome biology, i.e., interphase FISH (I-FISH) (Vorsanova et al. 2013). Since that time, no groundbreaking technological developments have been made in I-FISH or related techniques for studying interphase chromosomes. However, it seems that reconsidering technological aspects of interphase molecular cytogenetics is required, inasmuch as general decrease of interest to molecular cytogenetics (e.g., FISH) may be observed in the postgenomic era (Liehr 2017; Iourov 2019b; Heng 2020). Here we have reviewed I-FISH in the light of its application in the postgenomic context.

No fewer than one million cytogenetic and molecular cytogenetic analyses are suggested to be performed per year (Gersen and Keagle 2005). Molecular (cytogenetic) diagnosis is the standard of medical care for clinical genetics, reproduction, oncology, neurology, psychiatry, etc. (Vorsanova et al. 2010d; Bint et al. 2013; Liehr et al. 2015; Viotti 2020). The diagnostic value of FISH has been repeatedly noted and has been considered as either an alternative to conventional cytogenetic analysis or a confirmatory method (Feuk et al. 2006; Iourov et al. 2008c; Martin and Warburton 2015; Liehr 2017). In addition, I-FISH-like protocols are used in microbiology (Frickmann et al. 2017), genetic toxicology (Hovhannisyan 2010; Iurov et al. 2011), somatic cell genetics/genomics (Yurov et al. 2001, 2018b, 2019a; Iourov et al. 2008b, 2010b), aging research (Yurov et al. 2009, 2010a), and single-cell biology (Iourov et al. 2012, 2013a; Yurov et al. 2019b; Gupta et al. 2020). In summary, one can be certain that FISH-based molecular cytogenetic analysis has an important role in biomedicine.

In basic research, I-FISH is used for studying somatic chromosomal mosaicism (Iourov et al. 2006c, 2010a, 2017, 2019a, d; Arendt et al. 2009; Bakker et al. 2015; Andriani et al. 2019) and genome organization in interphase nuclei at the chromosomal level (Rouquette et al. 2010; Iourov 2012; Cui et al. 2016; Baumgartner et al. 2018). A successful study of the aforementioned phenomena requires the application of various I-FISH-based techniques, which are described in this chapter.

I-FISH

FISH is an umbrella term for molecular cytogenetic visualization techniques for studies of genome (specific genomic loci) using DNA/RNA probes. FISH resolution is defined by DNA sequence size of the probes. DNA probes are centromeric and telomeric (repetitive-sequence DNA), site-specific (euchromatic DNA, e.g., gene DNAs), and whole chromosome painting (wcp; hybridizing to the whole chromosomes DNAs) (Liehr et al. 2004; Iourov et al. 2008b; Vorsanova et al. 2013). Basically, I-FISH requires (i) cell suspensions prepared specifically for FISH analysis, (ii) denaturation of chromosomal DNA and hybridization, and (iii) microscopic visual and digital analysis of FISH results (Iourov et al. 2006b, 2017; Yurov et al. 2017).

FISH analysis of repetitive genomic sequences is performed with centromeric (chromosome enumeration or chromosome-specific). I-FISH with DNA probes for

repetitive sequences is applicable for analysis of nuclear chromosomal organization and numerical chromosome abnormalities (Yurov et al. 1996; Soloviev et al. 1998). I-FISH using centromeric DNA probes is used in molecular diagnosis (medical genetics, oncology, and reproduction) (Pinkel et al. 1986; Vorsanova et al. 1986, 2005b, 2010a; Yurov et al. 2007b, 2010b; Savic and Bubendorf 2016). Furthermore, I-FISH demonstrates these protocols highly applicable for studies encompassing chromosome biology, genome research (chromosomal and nuclear), evolution, behavior, and variation in health and disease (Liehr 2017). Near 100% hybridization efficiency and chromosome specificity (apart from chromosomes 5 and 19, 13 and 21, 14 and 22) defines I-FISH with these DNA probes as an effective molecular cytogenetic approach (e.g., analysis of homologous chromosomes in interphase) (Iourov et al. 2006d; Wan 2017; Russo et al. 2016; Yurov et al. 2017; Weise et al. 2019) (Fig. 7.1). I-FISH is shown to have the highest efficiency in uncovering mosaicism rates (Iourov et al. 2013b).

Site-specific DNA probes (yeast artificial chromosomes or YACS, bacterial artificial chromosomes or BACs, P1-derived artificial chromosomes or PACs, cosmids) provide the visualization of euchromatic chromosomal DNA. These probes are useful for targeted FISH assays to diagnose structural and, more rarely, numerical chromosome imbalances (Fig. 7.2) (Soloviev et al. 1995; Liehr et al. 2004; Riegel 2014; Cheng et al. 2017; Liehr 2017). The use of I-FISH assays with site-specific DNA probes is systematically applied in cancer research and molecular oncologic diagnosis (Chrzanowska et al. 2020). In the postgenomic era, these methods is applicable for mapping altered genomic loci, chromosome instability analysis, and arrangement of specific chromosomal loci in interphase.

I-FISH with chromosome-enumeration and site-specific probes may be affected by several phenomena occurring in interphase nuclei. Variable efficiency of hybridization complicates simultaneous applications of different probe sets, i.e., some signals can be invisible because of intensity differences (Iourov et al. 2006a). S phase DNA replication cause doubling of I-FISH signals (site-specific and centromeric probes) (Soloviev et al. 1995; Vorsanova et al. 2001a). False-positive chromosome abnormalities may be "uncovered" due to specific nuclear interphase chromosome architecture (genome organization). For instance, chromosomal associations affect I-FISH interpretation. Chromosomal associations/pairing are common in postmitotic cells types (Yurov et al. 2005, 2007a, 2008, 2014, 2018a; Iourov et al. 2009a, b). Quantitative FISH (QFISH) is used to differ between chromosome losses and chromosomal associations (discussed below). Solutions for these problems are given in Fig. 7.3. Finally, an appreciable increase of FISH efficiency may be achieved using microwave activation (for more details, see Soloviev et al. [1994], Durm et al. [1997], Weise et al. [2005]).

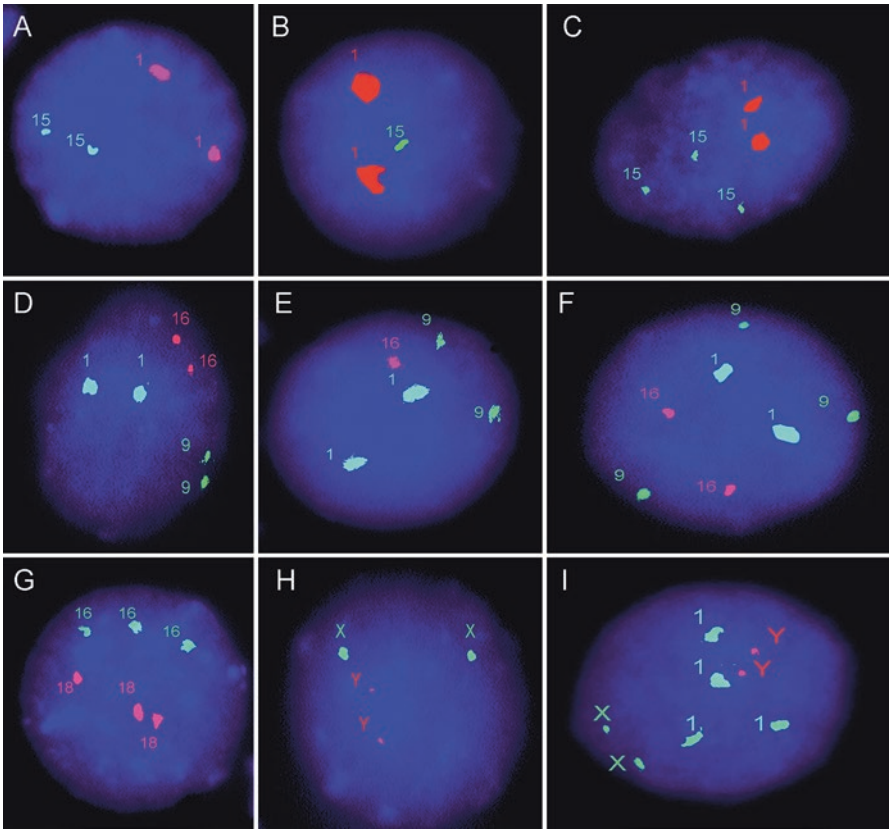

Fig. 7.1 Two- and three-color I-FISH with centromeric DNA probes. (**a**) Normal diploid nucleus with two signals for chromosome 1 and chromosome 15. (**b**) Monosomic nucleus with two signals for chromosome 1 and one signal for chromosome 15. (**c**) Trisomic nucleus with two signals for chromosome 1 and three signals for chromosome 15. (**d**) Normal diploid nucleus with two signals for chromosome 1, chromosome 9, and chromosome 16. (**e**) Monosomic nucleus with two signals for chromosome 1 and chromosome 9 and one signal for chromosome 16. (**f**) Trisomic nucleus with two signals for chromosome 1 and chromosome 16 and three signals for chromosome 9. (**g**) Triploid nucleus with three signals for chromosome 16 and chromosome 18. (**h**) Tetraploid nucleus with two signals for chromosome X and chromosome Y. (**i**) Tetraploid nucleus with two signals for chromosome X and chromosome Y and four signals for chromosome 1. ()

ICS-MCB

Microdissected DNA probes may be combined to produce pseudo-G banding using FISH or multicolor banding (MCB) (Liehr et al. 2002). This technique may be applied to interphase chromosomes in a chromosome-specific manner. Interphase chromosome-specific MCB (ICS-MCB) allow the visualization of interphase

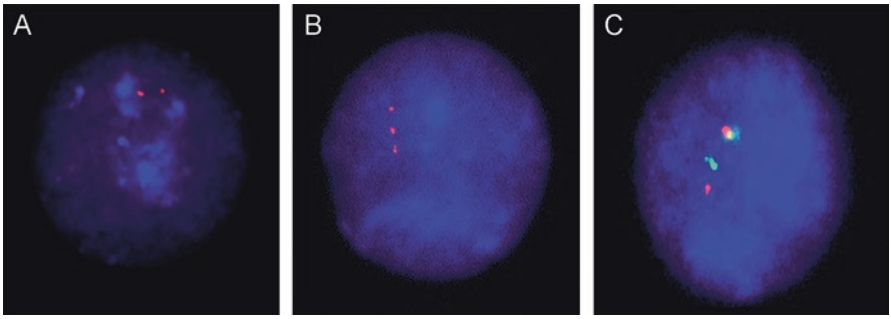

Fig. 7.2 I-FISH with site-specific DNA probes. (**a**) Normal diploid nucleus with two signals for chromosome 21. (**b**) Trisomic nucleus with three signals for chromosome 21. (**c**) Interphase nucleus exhibiting co-localization of *ABL* and *BCR* genes probably due to t(9;22)/Philadelphia chromosome. (Copyright © Vorsanova et al. 2010a; 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)

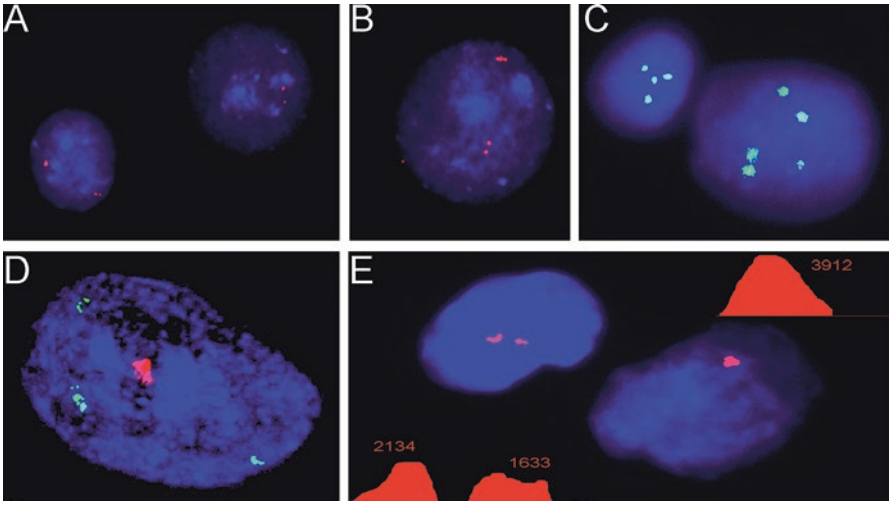

Fig. 7.3 Problems of I-FISH with centromeric/site-specific DNA probes. (**a**) and (**b**) Replication of specific genomic loci (LSI21 probe). Some nuclei exhibit replicated signals, whereas in some nuclei, it is not apparent. Note the distance between signals can be more than a diameter of a signal. (**c**) Asynchronous replication of a signal (DXZ1) in case of tetrasomy of chromosome X. Note the difficulty to make a definitive conclusion about number of signals in the right nucleus. (**d**) Two-color FISH with centromeric/site-specific DNA probes for chromosome 1 shows chromosomal associations in a nucleus isolated from the adult human brain. Note the impossibility to identify number of chromosomes. (**e**) QFISH demonstrating an association of centromeric regions of homologous chromosomes 9, but not a monosomy or chromosome loss. (Copyright © Vorsanova et al. 2010a; 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)

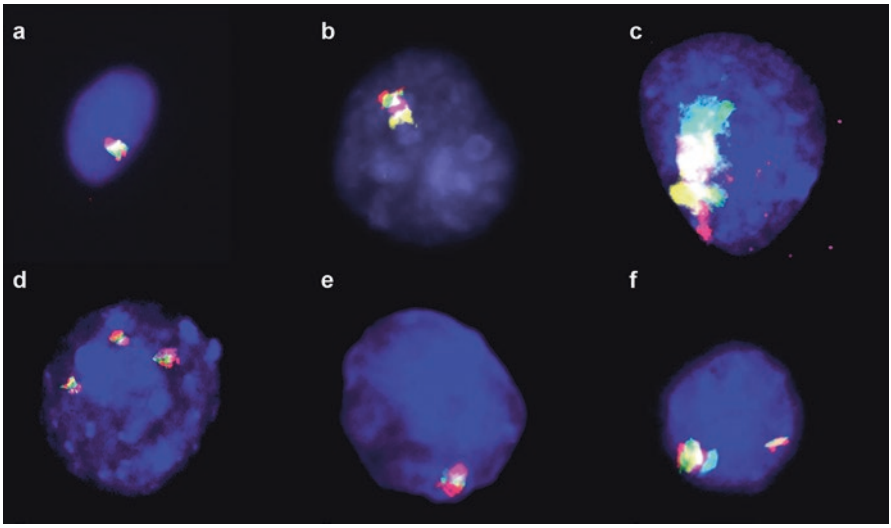

Fig. 7.4 Molecular cytogenetic analyses of the developing and adult human brain by ICS-MCB: (**a**) loss of chromosome 18 (monosomy) in a cell isolated from telencephalic regions of the fetal brain; (**b**) loss of chromosome 16 (monosomy) in a cell isolated from the cerebral cortex of the normal human brain; (**c**) loss of chromosome 1 (monosomy) in a cell isolated from the cerebral cortex of the schizophrenia brain; (**d**) gain of chromosome 21 (trisomy) in a cell isolated from the cerebral cortex of the Alzheimer's disease brain; (**e**) loss of chromosome 21 (monosomy) in a cell isolated from the cerebellum of the ataxia-telangiectasia brain; (**f**) chromosome instability in the cerebellum of the ataxia-telangiectasia brain manifesting as the presence of a rearranged chromosome 14 or der(14)(14pter- > 14q12:). (From Yurov et al. 2013 (Fig. 9.2) reproduced with permission of Springer Nature in the format reuse in a book/textbook via Copyright Clearance Center)

chromosomes in their integrity at molecular resolution (Iourov et al. 2006a, 2007). The method has been found highly effective for analysis of interphase chromosome instability and nuclear genome organization at chromosomal level (Iourov et al. 2006a, 2009a, b, 2019a; Yurov et al. 2007a, 2008, 2010b, 2014, 2019b; Liehr and Al-Rikabi 2019; Weise et al. 2019). Figure 7.4 gives a series of examples of ICS-MCB.

Immuno-FISH

Immuno-FISH is the combination of immunohistochemical detection of proteins and I-FISH (Liehr 2017). Our experience demonstrates that this technique is useful for studying chromosome instability in the human brain following by uncovering new mechanisms for neurodegeneration (Iourov et al. 2009a, b; Yurov et al. 2018b, 2019a). More precisely, immuno-FISH using NeuN antibody allows the detection of chromosomal DNA in neuronal cells (Fig. 7.5).

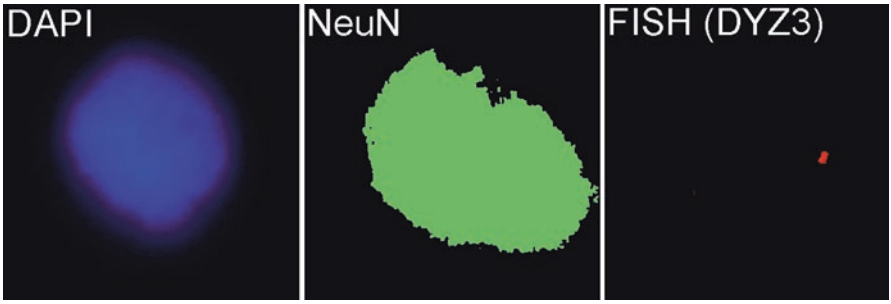

Fig. 7.5 Immuno-FISH**.** I-FISH using centromeric probe for chromosome Y (DYZ3) with immunostaining by NeuN (neuron-specific antibody) performed for the analysis of cells isolated from the human brain. (Copyright © Vorsanova et al. 2010a; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License, http://creativecommons.org/licenses/by/2.0)

QFISH

Interindividual variability of centromeric (heterochromatic) DNAs has been used of developing QFISH. This method is applicable for metaphase and interphase analysis of human chromosomes (Iourov et al. 2005; Vorsanova et al. 2005a; Iourov 2017). QFISH with chromosome-enumeration probes may be used for the detection of numerical imbalances of interphase chromosome (monosomy or chromosome loss). The latter is useful for prenatal and postnatal molecular diagnosis, cancer diagnosis and prognosis, and analysis of somatic genomic variability (Iourov 2017; Wan 2017; Yurov et al. 2017) (Fig. 7.6).

Molecular Diagnosis

An advantage of FISH-based techniques is referred to the availability of single-cell analysis (Iourov et al. 2012; Moffitt et al. 2016; Zhang et al. 2018). Despite the availability of DNA sequencing technologies for single-cell analysis (Knouse et al. 2014; Gawad et al. 2016), these cannot substitute FISH due to following reasons: FISH has the highest possible cell scoring potential and allows visualization of arrangement of genomic loci in interphase/metaphase chromosomes (Moffitt et al. 2016; Yurov et al. 2018b, 2019b). Accordingly, I-FISH is an important technique used in molecular cytogenetic diagnosis. Chromosomal imbalances cause a wide spectrum of diseases from congenital malformations, intellectual disability, autism, epilepsy, cancers, neurodegeneration, and reproductive problems (Vorsanova et al. 2001b, 2007, 2010b; Yurov et al. 2001, 2007b, 2019a, b; Gersen and Keagle 2005; Iourov et al. 2006c, 2008a, b, 2010b, 2011; Ye et al. 2019). Thus, the aforementioned FISH methods may be applicable for the molecular diagnosis. Since a diagnosis is aimed at uncovering molecular and cellular mechanisms for a disease, FISH

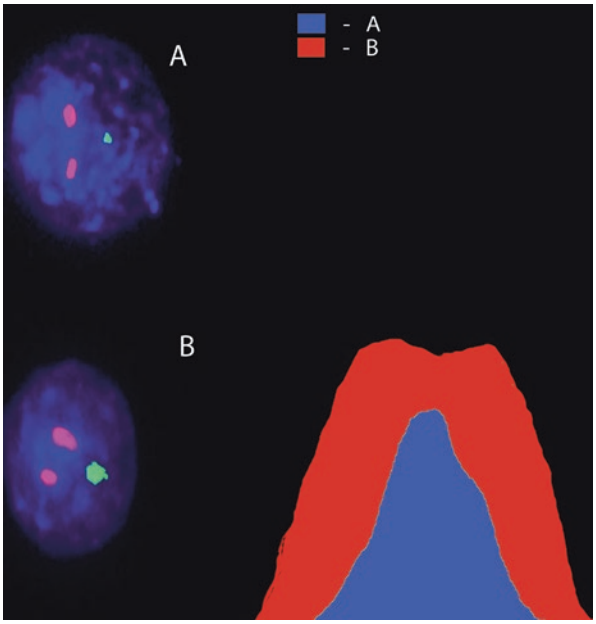

Fig. 7.6 QFISH with using enumeration-centromeric probes for chromosomes 1 (red signals/D1Z1) and X (green signals/DXZ1): Nucleus A demonstrates a green signal with a relative intensity of 2120 pixels—true X chromosome monosomy. Nucleus B demonstrates a green signal with a relative intensity of 4800 pixels—two overlapping chromosome X signals but not a chromosome loss. (From Yurov et al. 2017 reproduced with permission of Springer Nature in the format reuse in a book/textbook via Copyright Clearance Center)

should be considered as a technique additional to whole-genome analysis (e.g., whole-genome sequencing or molecular karyotyping) for uncovering processes, which are involved in the pathogenetic cascade of a disease (i.e., chromosome instability). The postgenomic era offers numerous possibilities for pathway-based classification of genome variations to model functional consequences of a genomic change. As a result, candidate processes may be suggested (Iourov 2019b; Iourov et al. 2019b, c). Currently, several bioinformatics tools are available for molecular cytogenetics (Iourov et al. 2012, 2014b; Zeng et al. 2012). Once applied, knowledge about mechanisms of disease mediated by chromosome abnormalities allows to propose successful therapeutic strategies for presumably incurable genetic conditions (Iourov 2016; Iourov et al. 2015b). Our experience of combination of whole-genome analysis (molecular karyotyping), I-FISH, and bioinformatics analysis is shown by Fig. 7.7 (Iourov et al. 2015a). Moreover, I-FISH analysis of chromosome inability may be integrated into molecular cytogenetic diagnostic workflows (Iourov et al. 2014a).

Taking into account promising biomarkers revealed by FISH, an algorithm for identifying disease mechanisms may be proposed. To succeed, two data sets are required: (1) cytogenetic/FISH data set (analysis of large cell populations for

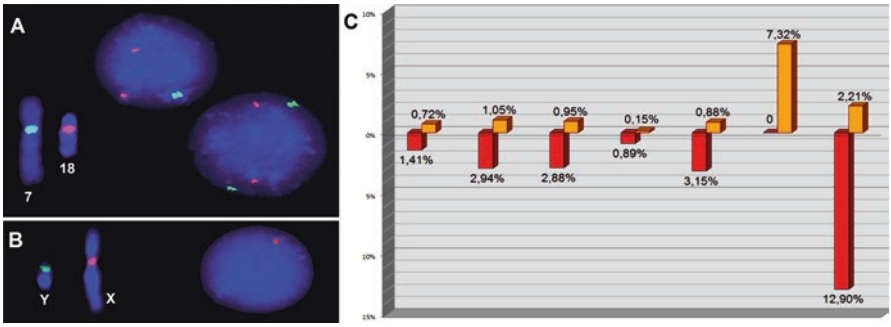

Fig. 7.7 Interphase FISH analysis of CIN (somatic aneuploidy). (**a**) FISH with DNA probes for chromosomes 7 (green) and 18 (red) showing chromosome 7 loss in the right nucleus (metaphase chromosomes show positive signals for these DNA probes). (**b**) Interphase FISH with DNA probes for chromosomes Y (green) and X (red) showing chromosome Y loss in the nucleus (metaphase chromosomes show positive signals for these DNA probes). (**c**) Rates of chromosome losses (red bars) and gains (golden bars). (From Iourov et al. 2015a, an article is distributed under the terms of the Creative Commons Attribution 4.0 International License, http://creativecommons.org/licenses/by/4.0/)

uncovering intercellular karyotypic variations) and (2) data set obtained by molecular karyotyping and analyzed using systems biology (bioinformatic) methodology for determining functional consequences of regular genomic variations. Once obtained, correlative analysis between these data sets is to be performed (Iourov 2019a; Vorsanova et al. 2019). Figure 7.8 reproduces this algorithm.

Conclusion

I-FISH seems to be an important technological part of current biomedical research and molecular diagnosis. Regardless of significant achievements in genomics and molecular biology, there is a wide spectrum of applications of this molecular cytogenetic technique. Mosaic chromosome abnormalities and chromosomal instability are relevant to numerous areas of biomedicine and require specific molecular cytogenetic approaches to the detection. Indeed, I-FISH-based techniques have to be included in the algorithms of detecting somatic genome variations at chromosomal and sub-chromosomal levels. In addition to detecting chromosomal mosaicism per se, I-FISH-based techniques are applicable to monitor somatic genomic changes and/or uncovering genome/chromosome insatiability, which may be either a cause of disease or an element of the pathogenetic cascade. Nuclear arrangement of chromosomes cannot be adequately addressed without I-FISH-based techniques. These studies are valuable for understanding genetic processes occurring in the interphase nucleus. Moreover, it is highly likely that exogenous influencing of chromosomal arrangement in interphase nuclei is a therapeutic opportunity for diseases associated with chromosomal imbalances, susceptibility to chromosome/genome instability,

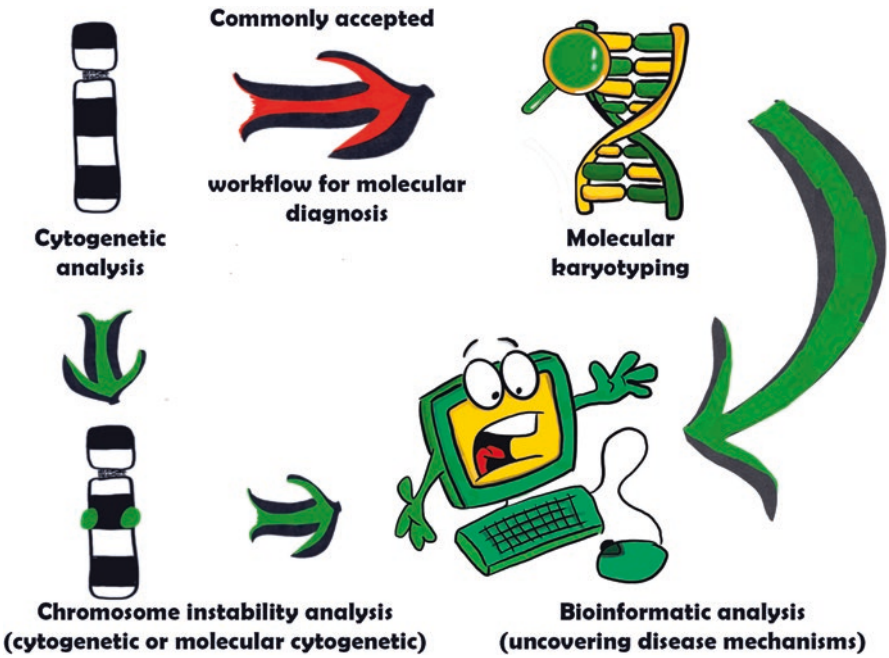

Fig. 7.8 Schematic depiction of the algorithm for investigating the molecular and cellular mechanisms of diseases mediated by CIN. To succeed, one has to follow green arrows or, in other words, to analyze chromosome instability by karyotyping and FISH (analysis of larger amounts of cells) instead of the commonly accepted workflow including only cytogenetic karyotyping and molecular karyotyping; bioinformatics is mandatory for uncovering disease mechanisms. ()

altered programmed cell death, and abnormal chromatin remodeling. In total, one can conclude that interphase molecular cytogenetics possesses actual methodology for basic and diagnostic research in genetics/genomics, cellular and molecular biology, and molecular (genome) medicine despite the availability of postgenomic technologies.

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