

Verification of the genomic identity of candidate microchimeric cells

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Microchimerism has been studied in the context of a variety of diseases which include autoimmune diseases (such as systemic sclerosis, rheumatoid arthritis, systemic lupus erythematosus and autoimmune thyroid diseases), cancer (e.g., of the cervix, thyroid gland, lung, breast), tissue repair, transplantation and transfusion. It may become relevant in the context of cell-based non-invasive prenatal diagnosis. But how to safely identify individual microchimeric cells? This is a nontrivial question, for which a solution has recently been suggested.

Verification of the genomic identity of chimeric cells, i.e., confirmation of fetal or maternal origin of the respective cells is the more difficult the lower the share of chimeric cells is in relation to the entirety of cells. This is important not only if we want to safely identify microchimeric cells in tissues in the context of research in autoimmune diseases,¹ in tissue repair and regeneration,² in cancer,³ transplantation and transfusion^{4,5} and pregnancy complications,⁶ but also of very practical relevance for the quest of cell-based non-invasive prenatal diagnosis, which relies decisively on safe identification of single cells which are part of the very small population of fetal cells present in the peripheral blood of pregnant women. In general, due to the problem of population overlap detection on the basis of biochemical parameters, even highly specific markers such as embryonic hemoglobin (Hb ϵ) expressed by embryonic nucleated red blood cells cannot lead to perfectly safe identification of rare single cells.⁷ The same is true for fluorescence in situ hybridization (FISH) which may yield false positive signals in

the percent range, consequently it is not reliable for safe identification of single rare cells either. Also, in comparison to immunocytochemical staining for biochemical markers it is more difficult to screen large numbers of cells for rare FISH-positive ones. FISH was optimized to fit rare cell conditions using two different Y probes⁸ and reverse XY-FISH,⁹ but the identification of fetal cells based on Y-FISH does not allow for a diagnosis in the case of female fetuses. The risk of contamination with host cells goes along with pooling of microchimeric cells.

Multiplex PCR using microsatellite loci is most promising as it allows for sex-independent identification of cells and, in combination, for molecular genetic diagnosis. We have developed a method using automated cell detection based on cells labeled on the basis of a biochemical marker with laser microdissection in combination with a slide screening application, allowing for the definition of candidate microchimeric cells. The sex-independent verification of the genomic identity of these genomically haplo-identical single rare cells is subsequently done by slide-based DNA typing (multiplex PCR using microsatellite loci plus analysis of the amelogenin gene which is located on both the X and Y-chromosome, albeit at different sizes). We suggest this procedure as a standard for sex-independent identification of fetal cells in the setting of rare cell analysis.¹⁰

Furthermore, we developed a method which avoids exhaustion of the available DNA for target cell identification and which allows for both genomic identification as well as for molecular genetic and cytogenetic analysis of the same cell. For this purpose we implemented a step of

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isothermal whole genome amplification (iWGA) in between the laser-microdissection of cells and the DNA typing. This way we accomplish the potentiality of performing multiple analyses from one particular cell by using aliquots of the iWGA amplicons, allowing for sex and cell-type independent identification of its genomic origin and in addition also analysis of specific genetic features.¹¹

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