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DESIGN OF A SPECIFIC DNA PROBE COCKTAIL FOR TARGETING BY FISH RET/PTC AND PAX8/PPARG REARRANGEMENTS IN THYROID TUMOR CELLS

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The precise diagnosis of a growing number of tumors, mostly relying on the histopathological conventional diagnosis, may today benefit from the use of fluorescent DNA molecular probes for fluorescence in situ hybridization (FISH) experiments. FISH probes are designed to target specific regions of DNA, and thus to elucidate abnormalities even at the gene level. Such molecules are able to reveal gene loss, gene amplification, and chromosome structural rearrangements leading to gene fusion, in a single cell. In case of translocation, two distinct FISH approaches are used, the dual-fusion FISH (with the probe set including DNA sequences encompassing proximally and distally the translocation breakpoints) and the break-apart FISH (in which the probe comprises DNA sequences mapped proximally and distally to the breakpoint within a critical gene). Most of these molecular probes are commercially available. Of course their production, following the market law, depends on the incidence of the disease for which a specific probe may be designed. For this reason, the need of DNA probes for research purposes requires laboratories to produce home-brew probes, which must be designed and validated, and eventually commercialized. Two subtypes of the most common differentiated thyroid tumors (papillary thyroid tumor - PTC - and follicular thyroid tumor - FTC) are associated with two distinct gene fusions: RET/PTC and PAX8/PPARg (Kondo et al., 2006). Recently, we started a project with the aim of designing a unique DNA break-apart probe cocktail, able to disclose, in a single FISH experiment, the presence of RET or PPARg disruption (and hence the gene fusions), as the result of the simultneous presence in the hybridization solution of four sets of probes each set labeled with fluorophores emitting distinguishable fluorescence signals. Here we report the first steps of the project: the online search for suitable BAC clones mapping distally and proximally to the breakpoints of the two genes, the confirmation of their chromosome localization on normal lymphocyte metaphases, and the preliminary preparation of two distinct probes cocktails, able to indicate RET/PTC or PAX8/PPARg gene fusions, respectively. Two BAC clones spanning 272,9 kb proximally to RET and two clones spanning 331,5 kb distally to RET, three BAC clones spanning 609.7 kb proximally to PPARg and three clones spanning 528,4 kb distally to PPARg were selected and expanded. The DNA was isolated and labeled by nick translation. The specificity of the 10 selected DNA probes was checked on metaphase chromosomes of a male with normal karyotype. The two cocktails were prepared by mixing the distal (labeled with Spectrum Green) and proximal (labeled with Spectrum Red) BACs, and were separately tested. As expected, the red and green signals were on chromosome 10 for probes targeting RET and on chromosome 3 for PPARg, and were adiacent or overlapping in interphase nuclei. On the contrary, the red and green signals were splitted in nuclei from positive controls, i.e. PTC with RET/PTC and FTC with PAX8/PPARg rearrangement tested by PCR. To achieve the final step of this research, we are evaluating the labeling of each probe set with ddUTP conjugated to different fluorophores. Such a mix could lead to the development and validation of a molecular commercial kit for targeting RET or PPARg breakage in a single FISH experiment, a poweful help especially for the pre-surgical diagnosis of thyroid nodules.

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