Preparation of human tumor samples for high-resolution DNA flow cytometry

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Background
Field cancerization is an accepted model for oral carcinogenesis. It is intended to give explanation to the development of oral cancer in multifocal areas of precancerous change and of the quite common presence of multiple independent lesions that sometimes coalesce. Similarly, the persistence of microscopically abnormal tissue after surgery might explain second primary tumors and local recurrences. Molecular findings further supported this model indicating that a more insidious field effect was because of genetic alterations not detectable by routine diagnostic technique, involving wide areas. Pentenero et al.¹-³ assessed molecular signs of chromosomal instability of the distant mirror fields with respect to oral leukoplakias.
This protocol describes the standard procedure used by Pentenero et al. to extract DNA from human tumor samples for subsequent high-resolution DNA flow cytometry (hr DNA FCM) using the gentleMACS™ Dissociator.

Materials and methods

Materials
- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS C Tubes
- Nylon mesh (50 µm mesh size)
- U-bottom tubes
- Shaker
- Refrigerated centrifuge
- Detergent solution (0.1 M citric acid, 0.5% Tween® 20)
- Staining solution (0.4M Na₂HPO₄, 5mM DAPI)

Methods
To perform DNA index determination, the reference normal DNA sample (for example, healthy donor lymphocytes) must be processed simultaneously to the sample to be analyzed.
1. Transfer a microbiopsy sample or a chopped biopsy (less than 0.5 g) of human tumor tissue to a gentleMACS C Tube containing 2 mL detergent solution.
2. Tightly close the C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
3. Run the gentleMACS Program A four times, for a total of 100 seconds.
4. After termination of the program, detach C Tube from the gentleMACS Dissociator and add 1 mL of detergent solution.
5. Run the gentleMACS Program A two times, for a total of 50 seconds.
6. Detach C Tube and spin at 1200 rpm for 5 minutes at 10 °C.
7. Transfer the sample in a U-bottom tube and shake tube continuously for 20 minutes at room temperature (RT).
8. Filter the obtained nuclei suspensions into a nylon mesh (50 µm) and wash with 1 mL of detergent solution.
9. Count the nuclei using a small volume of the sample properly diluted, to know the exact total number of nuclei for each sample.
10. Spin the sample at 1500 rpm for 15 minutes at 10 °C.
11. Discard the supernatant and resuspend the sample in detergent solution (add ⅔ of final sample volume stated as the volume needed to have 700.000 nuclei/mL). Shake continuously for 10 minutes at RT.
12. Add ⅓ of final sample volume of staining solution to sample.
13. Carry out hr DNA FCM after at least 15 minutes of incubation at 4 °C.
(Note: Samples remain stable for 24–48 hours at 4 °C.)
Results

Pentenero et al. report the presence of chromosome aberrations in non-dysplastic and visually normal appearing mucosa regions located in the mirror position and within the same oral subsite with respect to oral leukoplakias (fig. 1). These findings suggest that mirror fields relative to non-dysplastic and dysplastic leukoplakias were characterized by chromosomal instability.

![Histograms for the determination of the DNA content and the relative DNA Index (DI) using high resolution DNA FCM analysis. DI is calculated as ratio between the G0–G1 mean channel of the aneuploid population with respect to the G0–G1 mean channel of the diploid population. With this procedure for hDNA FCM analysis it can be distinguished as different two population that have a Δ of DNA content ≥2.5%. In the A and B panels are shown analyses of samples (a microbiopsy by curettage) derived, respectively, from a cheek leukoplakia and a cancer tongue. In both cases lymphocytes obtained by an healthy donor were used as reference (sample with diploid content have DI=1). Histograms A1 and B1 show diploid control references; A2 and B2 show patient’s samples, and in A3 and B3 are reported the analyses after the mixing of the each sample with the lymphocytes-reference (processed simultaneously) for the determination of the diploid peak. Histogram A3 reported an example of the determination of an ipodiploid aneuploid population (DI<1), while in the histogram B3 the aneuploid population is near-diploid iperdiploid (1<DI<1.4).]
Conclusion

DNA extraction from human tumor samples for subsequent hr DNA FCM can be accomplished with ease using the gentleMACS Dissociator.

References