Efficiency in recovery of pure tumor cell populations from limited tumor tissue specimens intended for clinical application

Valeria Sero (1), Claudio Forcato (2), Chiara Bolognesi (2), Genny Buson (2), Giulio Signorini (2), Paola Tonori (2), Gianni Medoro (2), Nicolò Manaresi (2), Farideh Z. Bischoff (1),
(1) Menarini Silicon Biosystems Inc, San Diego, CA (2) Menarini Silicon Biosystems SpA, Bologna, ITALY

Results

DEPArray analysis allowed identification of well separated cell populations, including Tumor (Vim+/Ker+) and Stromal (Vim+/Ker−) cells in all the samples analyzed. Among the Microdissected samples we were able to estimate % of tumor cells (% of V+K− events) ranging from 22% to 44% (mean 34%), demonstrating an unexpected low frequency of tumor cells remaining following macrodissection. Among FFPE samples analyzed only 21% (4.2% to 47.2% range) of the total (mean of 633) cells analyzed were of tumor (Vim+K−) origin. For subsequent NGS analysis, groups of pure cells (mean 214 cells, range from 37 to 280) for each population were recovered. Among the tumors isolated from the Microdissected and FNA samples, we observed non-synonymous somatic variants and LOH events for different genes. These events can not be detected in unsorted populations. In fact, only in the tumor cells isolated from the Microdissected breast cancer specimen, we observed three homozygous somatic variants (FOXP1, PTEN and PIK3CA), all annotated in COSMIC database and with missense/stop-gain effect in the coding sequence of related genes. However, the unsorted (prior to DEPArray) population shows a frequency of approximately 30% for all three variants, while only the wild type allele is present in the recovered pure stromal cells. This implies that the unsorted population contains the wild-type allele, coming from the stromal cells. The amount of this allele contamination is approximately 70%, consistent with DEPArray analysis. While in one of the breast cancer FNA samples, the TP53 loss of heterozygosity was observed but only in the sorted tumor (Vim+) cells and not in the unsorted, stromal (Vim−) cells. In addition a PIK3CA missense somatic heterozygous variant was detected in both tumor and EMT populations but notably was absent in stromal cells confirming it as a somatic mutation.

Conclusions

DEPArray™ can be used to isolate pure tumor cells from heterogeneous tumors limited in tumor content that were obtained through multiple procedures such as FFPE macrodissection. Our approach brings precision to detection, quantification and recovery of pure target cells from even the most unexpected specimens (FNA) that are now suitable for downstream molecular analysis.

效率在有限的肿瘤组织样本中获得纯肿瘤细胞群体

瓦莱里亚·塞罗（1）、克劳迪奥·福卡托（2）、奇拉·博洛内西（2）、盖尼·布松（2）、朱利欧·西格诺里尼（2）、帕奥拉·托诺里（2）、吉安尼·梅多罗（2）、尼科洛·马纳雷西（2）、法里德·Z·比什乔夫（1），
（1）梅纳里尼硅生物系统公司，圣地亚哥，CA （2）梅纳里尼硅生物系统公司，博洛尼亚，意大利

结果

DEPArray分析允许识别出良好分离的细胞群体，包括肿瘤（Vim+/Ker+）和间质（Vim+/Ker−）细胞。在所有样本中均进行了分析。在显微镜下分离的样本中，我们能够估计出肿瘤细胞的百分比（% V+K−事件）范围从22%到44%（平均34%），显示了未预期的低频率肿瘤细胞，出现在分离后。在从显微镜下分离和FNA样本中，我们观察到非同义体细胞变异和LOH事件的多种基因。这些事件在未分离的细胞中无法被检测到。只有在肿瘤细胞中分离的来自显微镜下分离的乳腺癌样本，我们观察到三个纯合体体细胞变异（FOXP1、PTEN和PIK3CA），所有在COSMIC数据库中被标注，并在相关基因的编码区有错义/终止-获得效应。然而，未分选（用于DEPArray）的群体中显示约30%的频率为所有三个变异，而仅野生型等位基因在分离的间质细胞中出现。这表明未分选的群体包含野生型等位基因，来自间质细胞。该变异的含量为约70%，与DEPArray分析一致。而在乳腺癌FNA样本中，TP53丢失的杂合性只出现在分离的肿瘤（Vim+）细胞中，而未在未分离的间质（Vim−）细胞中。此外，PIK3CA体细胞等位基因突变在肿瘤和EMT群体中被检测，但在间质细胞中未被检测，证实这是一个体细胞突变。

结论

DEPArray™可用于分离纯肿瘤细胞，从有限的肿瘤组织中获得，这些组织被通过多种程序，如FFPE显微镜下分离。我们的方法提高了检测、量化和分离纯目标细胞的能力，甚至对最少的未预期样本（FNA），这些样本现在适合用于下游分子分析。

© Menarini Silicon Biosystems SpA