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The examination of BRC markers expression and the target sequence amplification for single MTSs

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MTS

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Abstract

The examination of BRC markers expression and the target sequence amplification for single MTSs.

Guidelines

The BRC tissues and adjacent tissues underwent sequence-specific amplification using a primer pool comprising the key 17 mutation sites in BRC genes listed in Data File S4. Total RNA was extracted using the Trizol kit (Thermo Fisher Scientific). Subsequently, reverse transcription (RT) was performed with 0.5 μg purified RNA using the HiScript III 1st Strand cDNA Synthesis Kit (+gDNA wiper) (Vazyme). Following PCR reaction using cDNA as template, the specific fragments were subjected to Sanger sequencing (GENEWIZ) for the examination of mutations in amplified cDNAs.

Materials

Trizol kit (Thermo Fisher Scientific), HiScript III 1st Strand cDNA Synthesis Kit (+gDNA wiper) (Vazyme), ChamQ Universal SYBR qPCR Master Mix (Vazyme), LightCycle480 II (Roche), TaKaRa Taq DNA Polymerase, PrimerBank software, GraphPad software (Version 10), Single Cell Sequence Specific Amplification Kit (Vazyme), CytoSTAR apparatus (Livingchip), T100™ (Bio-rad), Sanger sequencing (GENEWIZ)

Examination of BRC markers expression through real-time PCR (qPCR) analysis

1 Total RNA was extracted from pooled MTSs, RCs on MWC-chips, BRC and adjacent tissues using the Trizol kit (Thermo Fisher Scientific). Subsequently, reverse transcription (RT) was performed with 0.5 µg purified RNA using the HiScript III 1st Strand cDNA Synthesis Kit (+gDNA wiper) (Vazyme).

2 The expression levels of BRC marker genes, including CD44, CD24, ALDH1A1, c-MYC, NANOG, SOX2, CXCR4, BMI, as well as lymphocyte marker CD45 and fibroblast marker FAP were quantified using qPCR with ChamQ Universal SYBR qPCR Master Mix (Vazyme) on the LightCycle480 II (Roche).

3 A 10 µL PCR reaction mixture containing 0.5 µL diluted cDNA, primers, dNTPs, and TaKaRa Taq DNA Polymerase was used. The PCR reaction consisted of an initial denaturation step at 94 °C for 3 minutes followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 68 °C for 30 seconds and extension at 72 °C for one minute.

4 The specific primer sequences were designed using PrimerBank software and are listed in Data File S4. Relative mRNA levels were determined by normalizing to GAPDH mRNA levels (used as an internal control) using the 2^-ΔΔct method in GraphPad software (Version 10, GraphPad Software).

Target sequence amplification for single MTSs

5 RNA extraction, reverse transcription, and amplification were performed on single MTS cells using a Single Cell Sequence Specific Amplification Kit (Vazyme) following the manufacturer's instructions. Briefly, single MTSs were picked up from the MWC-chips using CytoSTAR apparatus (Livingchip) and transferred to a 200 µL tube containing 8 µL of Reaction buffer mixed with a Primer Pool at a concentration of 0.1 µM and RT/Taq enzyme.

6 After a 17-cycle reaction (T100™, Bio-rad), the resulting cDNA was diluted and prepared for subsequent qPCR reactions to assess the expression levels of BRC marker genes, or for examination of site-specific mutations in BRC genes.

Examination of site-specific mutations in BRC and adjacent tissues

7 The BRC tissues and adjacent tissues underwent sequence-specific amplification using a primer pool comprising the key 17 mutation sites in BRC genes listed in Data File S4. Total RNA was extracted using the Trizol kit (Thermo Fisher Scientific). Subsequently, reverse transcription (RT) was performed with 0.5 µg purified RNA using the HiScript III 1st Strand cDNA Synthesis Kit (+gDNA wiper) (Vazyme). Following PCR reaction using

cDNA as template, the specific fragments were subjected to Sanger sequencing (GENEWIZ) for the examination of mutations in amplified cDNAs.