Author’s response to reviews

Title: Characterization of human mesothelin transcripts in ovarian and pancreatic cancer

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We appreciate the thorough review of the manuscript and have addressed the reviewer's concern as follows.

We agree that accuracy in the sequence analysis represents a critical point in the manuscript. To this end, a total of nine independent PCR products from seven different sources were sequenced, including products from four cell lines, one normal ovarian tissue sample and two ovarian tumor samples. Additionally, an alternative set of amplification primers was used on two samples (Hela and one ovarian tumor), and gave identical results. DNA sequence analysis was performed using fluorescence-based cycle sequencing and the ABI BigDye Terminator V 3.1 Cycle Sequencing kit with the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). This instrument is fully automated and uses capillary electrophoresis and laser-assisted detection to limit day to day, and person to person variations. Accuracy is achieved by the instrument software and robust basecaller algorithms that accurately and reproducibly identify each base in the sequence. Typical sequence reads are 700 bp long with >98.5% basecalling accuracy. In all cases, the sequence in question fell well within an unambiguous region of basecalling. This technology contrasts with the older technology that likely produced the initially reported mesothelin sequence. Traditional sequencing methods using radioactive dideoxynucleotides rely on visual inspection of radioactive bands that can be distorted or irregular due to suboptimal gel conditions. This could potentially result in bands that are ambiguous leading to inaccurate readings. Based on the improved technology, identical results over multiples samples, and identity of the transcript sequence with reported genomic and expressed sequence databases, we feel confident that the sequences reported in the manuscript are accurate.

We have added additional information regarding the technology used to obtain the sequence data to the Methods section on page 9, and have more clearly delineated the PCR products sequenced in the Results section on page 12.