Quantification of alternatively spliced FGFR2 RNAs using the RNA invasive cleavage assay. [1]

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Abstract	The regulated splicing of fibroblast growth factor receptor-2 (FGFR2) transcripts leads to tissue-specific expression of distinct receptor isoforms. These isoforms contain two different versions of the ligand binding Ig-like domain III, which are encoded by exon IIIb or exon IIIc. The mutually exclusive use of exon IIIb and exon IIIc can be recapitulated in tissue culture using DT3 and AT3 rat prostate carcinoma cells. We used this well-characterized system to evaluate the precision and accuracy of the RNA invasive cleavage assay to specifically measure FGFR2 alternative splicing outcomes. Experiments presented here demonstrated that the RNA invasive cleavage assay could specifically detect isoforms with discrimination levels that ranged from 1 in 5 x 10(3) to 1 in 10(5). Moreover the assay could detect close to 0.01 amole of FGFR2 RNAs. The assay detected the expected levels of transcripts containing either exon IIIb or IIIc, but, surprisingly, it detected high levels of IIIb-IIIc double inclusion transcripts. This finding, which has important implications for the role of exon silencing and of mRNA surveillance mechanisms, had been missed by RT-PCR. Additionally, we used the RNA invasive cleavage assay to demonstrate a novel function for the regulatory element IAS2 in repressing exon IIIc inclusion. We also show here that purification of RNA is not necessary for the invasive cleavage assay, because crude cell lysates could be used to accurately measure alternative transcripts. The data presented here indicate that the RNA invasive cleavage assay is an important addition to the repertoire of techniques available for the study of alternative splicing.
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