High throughput screens for genes and compounds that block cancer progression and metastasis through changes in alternative splicing of proto-oncogene

Published date: Feb. 1, 2012

Technology description

Summary

Development of highly sensitive Luciferase-based alternative splicing reporter assay for use in high throughput screens for regulatory proteins and compounds that regulate splicing

Description

Our team has developed a series of fluorescence- and luciferase-based splicing reporter constructs that can be used to carry out high throughput functional assays for proteins or compounds that influence specific alternative splicing pathways ((Hovhannisyan et al., 2006; Newman et al., 2006)and submitted). The minigenes used in these assays were designed in a manner in which fluorescent or luciferase fusion proteins are encoded by a construct that contains introns and one or more exons whose inclusion or skipping determines a reading frame that will or will not yield a luciferase-positive product (Figure 1A). Therefore, increases (or decrease) in exon splicing can be detected in single wells of 386 well plates with high sensitivity using luciferase assays (Figure 1B). In control assay development experiments, this system demonstrated Z-scores of greater than 0.8 in screening conditions making it suitable for high throughput screening. The minigenes can be either transiently or stably expressed in cell lines for use in array-based format. These cells can thus be used to screen for changes in splicing induced in response to exogenously introduced cDNAs, interfering RNAs, or compounds including small molecules. Changes alternative splicing of numerous gene transcripts have been implicated in cancer development and progression (Hu and Fu, 2007; Venables, 2006). In a number of cases this has been shown to be due to changes in the expression or activity of splicing regulators that induce changes in splicing of transcripts encoding proto-oncogenes or tumor suppressors (Hu and Fu, 2007; Karni et al., 2007). Thus, there is a growing interest in the development of therapies that target specific changes in alternative splicing as well as the expression of genes that encode regulators of these splicing events. Indeed, use of fluorescent splicing reporters to carry out small molecule screens for compounds that affect splicing have recently been described (Stoilov et al., 2008). However, in our experience, use of luciferase-based assays is superior to a fluorescent readout due to much greater dynamic range of the

assay. These minigenes and reporter assays have already been functionally validated for use in high throughput cDNA expression screen to identify genes that regulate splicing of Fibroblast Growth Factor Receptor 2 (FGFR2), CD44, and hMena (ENAH); genes in which changes in alternative splicing have been implicated in the epithelial mesenchymal transition and cancer progression (Carstens et al., 1997; Pino et al., 2008; Ponta et al., 2003). In a submitted manuscript, we show that these assays led to the identification of two paralogous splicing regulatory proteins that we named Epithelial Splicing Regulatory Proteins 1 and 2 (ESRP1 and ESRP2). Expression of these proteins was shown to be necessary and sufficient for the expression of the epithelial variants of FGFR2, CD44, and hMena suggesting that they regulate a broad post-transcription expression program that is essential for epithelial differentiation. Although there is some controversy regarding the relationship between the EMT and tumor metastasis, there are numerous demonstrations that the EMT is one mechanism that can contribute to the metastatic process (Chaffer et al., 2007; Yang and Weinberg, 2008). A switch in FGFR2 splicing from the epithelial to menchymal splice variant of FGFR2 occurs during the EMT and expression of the mesenchymal isoform was recently shown to correlate with invasiveness in breast cancer cell lines (Cha et al., 2008). Our work demonstrated that expression of ESRP1 and ESRP2 is lost during the EMT in several cancer models, suggesting that therapies to restore their expression may prove useful in cancer therapies. These therapies would be predicted to affect splicing of known targets of the ESRPs and therefore could be identified using the splicing assay to screen for such compounds or therapies. These luciferase based assays can be easily adapted to screen for compounds that regulate FGFR2, CD44, and hMena splicing. However, there are also numerous other examples of alternative splicing that have been shown to play a role in tumorigenesis and cancer progression. The minigene reporters we have developed are modular and thus can be further adapted to develop screens for other cancer-related alternatively spliced exons for target assays.

Institution

University of Pennsylvania

联系我们

叶先生

电 话：021-65679356

手 机：13414935137

邮 箱：yeyingsheng@zf-ym.com