[0006] There is therefore a need to develop cell systems capable of high levels of HBV replication so as to provide a more sensitive testing system for the screening of HBV therapeutics.

SUMMARY

[0007] In one aspect, there is provided a cell comprising: a nucleotide sequence encoding a Hepatitis B Virus (HBV) operably linked to a promoter; two nucleotide sequences each encoding an isoform of HNF4α; and a nucleotide sequence encoding a repressor of HBV transcription, wherein said nucleotide sequence is mutated to decrease or silence expression of the repressor.

[0007a] In another aspect, there is provided a cell comprising: a nucleotide sequence encoding a Hepatitis B Virus (HBV) operably linked to a promoter; two nucleotide sequences each encoding a different isoform of HNF4α and a nucleotide sequence encoding a repressor of HBV transcription, wherein said nucleotide sequence is mutated to decrease or silence expression of the repressor.

[0008] In another aspect, there is provided a kit comprising the cell described herein together with instructions for use.

[0009] In another aspect, there is provided a method to produce HBV *in vitro* comprising culturing the cell described herein in the presence of an inducer for regulating transcription of the promoter.

[0010] In another aspect, there is provided a method of detecting the amount of HBV in a culture media *in vitro* comprising: culturing the cell described herein in a culture media comprising an inducer for regulating transcription of the promoter; contacting the cell with a probe capable of hybridizing to a target sequence on the HBV genome; hybridizing the probe to the target sequence, wherein a signal is emitted when the probe hybridizes to the target sequence; measuring the level of the emitted signal and comparing this to a signal from a reference sample to detect the amount of HBV in the culture media.

[0011] In another aspect, there is provided a method of identifying a HBV therapeutic agent comprising: culturing the cell described herein in a culture media comprising an inducer for regulating transcription of the promoter and the therapeutic agent; contacting the cell with a probe capable of hybridizing to a target sequence on the HBV genome; hybridizing the probe to the target sequence, wherein a signal is emitted when the probe

hybridizes to the target sequence; measuring the level of the emitted signal and comparing this to a signal from a reference sample, wherein a decrease in the emitted signal compared to the reference sample identifies the HBV therapeutic agent.

5

Claims

1. A cell comprising:
   a nucleotide sequence encoding a Hepatitis B Virus (HBV) operably linked to a promoter;
   two nucleotide sequences each encoding ~~an~~ a different isoform of HNF4α and a nucleotide sequence encoding a repressor of HBV transcription, wherein said nucleotide sequence is mutated to decrease or silence expression of the repressor.

2. The cell according to claim 1, wherein the HBV is of genotype B.

3. The cell according to claims 1 or 2, wherein the promoter operably linked to the nucleotide sequence encoding the HBV is an inducible promoter.

4. The cell according to claim 3, wherein the inducible promoter is a doxycycline inducible promoter.

5. The cell according to any one of claims 1 to 4, wherein the nucleotide sequence encoding HBV operably linked to a promoter is stably integrated into the genome of the cell.

6. The cell according to any one of claims 1 to 5, wherein the isoform of HNF4α is selected from the group consisting of HNF4α1, HNF4α2, HNF4α3, HNF4α4, HNF4α5, HNF4α6, HNF4α7, HNF4α8, HNF4α9, HNF4α10, HNF4α11 and HNF4α12.

~~7. The cell according to any one of claims 1 to 6, wherein each of the two nucleotide sequences encodes the same isoform, or different isoforms of HNF4α.~~

~~8.~~7. The cell according to any one of claims 1 to ~~7~~6, wherein the two nucleotide sequences encode isoforms HNF4α1 and HNF4α2 (HNF4α1-2), HNF4α2 and HNF4α3 (HNF4α2-3), HNF4α3 and HNF4α4 (HNF4α3-4), HNF4α2 and HNF4α6 (HNF4α2-6), HNF4α3 and HNF4α8 (HNF4α3-8), HNF4α4 and HNF4α8 (HNF4α4-8), HNF4α4 and HNF4α9 (HNF4α4-9), HNF4α6 and HNF4α12 (HNF4α6-12).

~~9.~~8. The cell according to any one of claims 1 to ~~7~~6, wherein the two nucleotide sequences encode isoforms HNF4α1 and HNF4α2 (HNF4α1-2).

~~10.~~9. The cell according to any one of claims 1 to ~~9~~8, wherein the mutation of the nucleotide sequence encoding a repressor of HBV transcription is selected from the group consisting of insertion, deletion, substitution or a combination thereof of one or more nucleotides.

~~11.~~10. The cell according to any one of claims 1 to ~~10~~9, wherein the repressor of HBV transcription is SLUG.

~~12.~~11. The cell according to claim ~~11~~10, wherein the nucleotide sequence encoding SLUG is mutated at one or more positions encoding amino acid residues starting from position 56 of SLUG.

~~13.~~12. The cell according to any one of claims 1 to ~~12~~11, wherein the nucleotide sequence encoding a repressor of HBV transcription is mutated by a CRISPR-Cas9 system.

~~14.~~13. The cell according to any one of claims 1 to ~~13~~12, wherein the cell is a hepatic cell.

~~15.~~14. The cell according to any one of claims 1 to ~~14~~13, wherein the cell is selected from the group consisting of HepG2, HuH7 and Hep3B.

~~16.~~15. The cell according to claim ~~15~~14, wherein the cell is HuH7.

~~17.~~16. The cell according to any one of claims 1 to ~~16~~15, wherein the cell is a cell line.

~~18.~~17. A kit comprising the cell according to any one of claims 1 to ~~17~~ 16 together with instructions for use.

~~19.~~18. A method to produce HBV *in vitro* comprising culturing the cell according to any one of claims 1 to ~~17~~ 16 in the presence of an inducer for regulating transcription of the promoter.

~~20.~~19. The method of claim ~~19~~18, wherein the HBV is produced at an increased level compared to a baseline level.

~~21.~~20. A method of detecting the amount of HBV in a culture media *in vitro* comprising:
culturing the cell of any one of claims 1 to ~~17~~ 16 in a culture media comprising an inducer for regulating transcription of the promoter;
contacting the cell with a probe capable of hybridizing to a target sequence on the HBV genome;
hybridizing the probe to the target sequence, wherein a signal is emitted when the probe hydridizes to the target sequence;
measuring the level of the emitted signal and comparing this to a signal from a reference sample to detect the amount of HBV in the culture media.

~~22.~~21. The method of claim ~~21~~20, wherein the inducer is doxycycline and wherein the promoter is inducible by a Tet-on system.

~~23.~~22. The method of claims ~~21~~ 20 or ~~22~~21, wherein the probe comprises a nucleotide sequence that is complementary to the target sequence on the HBV genome.

~~24.~~23. The method of any one of claims ~~21~~ 20 to ~~22~~21, wherein the probe further comprises a detectable label at the 5' end of the probe and a quencher on the 3' end of the probe.

~~25.~~24. The method of any one of claims ~~21~~ 20 to ~~24~~23, wherein the reference sample is a cell that does not produce HBV.

~~26.~~25. A method of identifying a HBV therapeutic agent comprising:
culturing the cell of any one of claims 1 to ~~17~~ 16 in a culture media comprising an inducer for regulating transcription of the promoter and the therapeutic agent;
contacting the cell with a probe capable of hybridizing to a target sequence on the HBV genome;
hybridizing the probe to the target sequence, wherein a signal is emitted when the probe hybridizes to the target sequence;
measuring the level of the emitted signal and comparing this to a signal from a reference sample, wherein a decrease in the emitted signal compared to the reference sample identifies the HBV therapeutic agent.

~~27.~~26. The method of claim ~~26~~25, wherein the inducer is doxycycline and wherein the promoter is inducible by a Tet-on system.

~~28.~~27. The method of claims ~~26~~ 25 or ~~27~~26, wherein the reference sample is a cell that has been cultured in media that does not comprise the therapeutic agent.