cryopreserved cultures. However, somatic cells isolated from tissues/organs often used in food consumption (e.g. muscle, fat, and fibroblasts) from agriculturally relevant species (e.g. pigs, cows, chickens) have a limited lifespan when grown in vitro. Although it is possible to isolate primary cell lines from pigs (myoblasts, myofibroblasts, fibroblasts, adipose derived stem cells and epithelial cells) the ability to propagate these cell lines with efficient doubling times and for long term is not feasible.

Merlin ((Moesin-ezrin-radixin-like protein, also known as schwannomin) is a tumour suppressor protein encoded by the neurofibromatosis type 2 gene *NF2*. The NF2 gene and the Merlin protein have a central role in a multitude of important developmental signalling pathways. For example, NF2/Merlin is a known activator of the Hippo pathway, which restricts organ sizes (Hamaratoglu *et al*., 2006 (doi:10.1038/ncb1339)) and plays a role in anoikis (Zhao et al, 2012; doi: 10.1101/gad.173435.111). NF2/Merlin has also been shown to modulate Ras activity in mice cells (Cui *et al*., 2019 (doi:10.1038/s41388-019-0883-6)). Furthermore, it is involved in WNT/β-catenin signalling, receptor tyrosine kinase signalling and NOTCH signalling pathways amongst others (Mota & Shevde 2020 (doi.org/10.1186/s12964-020-00544-7)). NF2 also plays a driving role in allowing cancer cells to escape the primary tumour niche, through downregulating cell-to-cell adhesions and thus promoting metastasis (Lallemand et al, 2003; doi: 10.1101/gad.1054603). NF2 knock-out models in various mice and human cell lines have shown increased cell proliferation and loss of contact inhibition (Wahiduzzaman *et al*., 2018 (doi:10.1111/cas.13871); Bosco et al 2010 (doi:10.1038/onc.2010.20); and Fomicheva & Macara, 2020 (doi:10.7554/eLife.636032).

There is a need to reduce cell doubling times (also in the context of suspension culture) and improve the economic viability of cultured meat. The invention addresses this need by providing manipulated primary animal cells that comprise modification of endogenous genes and do not require the expression of exogenous nucleic acid constructs. For this, the relative impact of loss of Hippo pathway members and regulators on proliferation in an anchorage-dependant and independent manner in various cell types and genetic backgrounds is compared.

Summary

In a first aspect of the invention, there is provided a cultured animal cell having a genetic modification in the NF2 gene or a modification to the activity of the Merlin protein and wherein the animal is of an animal species suitable for human or animal consumption.

In an example, there is provided a cultured animal cell wherein expression of the NF2 gene is modified or the activity of the Merlin protein is modified and wherein the animal is of an animal species suitable for human or animal consumption. It is described here for the first time that a functional knock-out of endogenous NF2 decreases doubling times in porcine and bovine myoblasts and Adipose-derived stem cells. Additionally, it is shown that alteration of NF2 supports the suspension adaptation process of adherent porcine and bovine cell cultures, which is essential to produce agriculturally relevant cell types for consumption. It is therefore advantageously demonstrated that inactivation of the endogenous NF2 gene is sufficient to speed up proliferation of porcine and bovine cell cultures and supports the suspension adaptation process. The commercial viability of cultured meat is greatly

improved by the aforementioned advantages of increased proliferation, i.e. decreased doubling time, and supporting the suspension adaptation process.

In one embodiment, the modification decreases the doubling time of the cell by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

In one example, the animal is selected from a pig, bovine, poultry, sheep, goat, Equidae, Camelidae, fish, crustaceans or mollusc.

In one example, the animal cell is a somatic cell.

In one example, the animal cell is selected from one of the following cell types: myoblast, fibroblast, myofibroblast, adipose derived stem cell, epithelial cell, mesenchymal stem cell, satellite cell, iPSC, or hepatocyte.

In one example, the expression of the *NF2* gene or the activity of the Merlin protein is modified in the animal cell by any one or more of:

1) gene level modification by:
    a. knock-out or reduced activity/transcription/translation levels via editing in coding sequences, promoters, introns, regulatory regions;
    b. RNA-directed DNA methylation; or
    c. transcription activation or repression using CRISPRa or CRISPRi or similar target specific methods;
    d. knock-out or reduced activity/transcription/translation levels via undirected means, for example radiation or chemical mutagenesis;
2) post-transcription level (post-transcriptional gene silencing) modification by:
    a. RNAi or siRNA to reduce translation of mRNA into protein; or
    b. site specific nucleases to modify or cleave mRNA, such as CRISPR/Cas13a;
3) post-translational level (protein disruption) modification by:
    a. inclusion of activity blocking/reducing molecules, wherein the activity blocking/reducing molecules are small molecules, antibodies, or the like; or
    b. inclusion of protein degrading ingredients, wherein the protein degrading ingredients are specialised proteases, exoproteases, or endoproteases.

In one example, the animal cell has a genetic modification in *NF2* gene.

In one example, the genetic modification in the *NF2* gene is a loss of function modification or leads to reduction in function..

In one example, the loss of function modification comprises a knock-out of the gene or loss of protein function.

In one example, the modification is introduced using targeted genome modification or randomised mutagenesis or by spontaneous mutation.

In one example, the modification is in the promoter region or coding region of the one of more genes.

In one example, the modification is introduced using targeted genome modification, optionally using a targeted endonuclease.

In one example, the endonuclease is optionally selected from TALEN, ZFN or CRISPR, optionally CRISPR/Cas9.

In one example, the cultured animal cell further comprises at least one additional genetic modification to manipulate the genome surveillance, cell cycle control and/or cell death control pathway.

In one example, the at least one additional genetic modification is in one or more of the following genes: RB1, TP53, and/or a RAS gene. In one example, the additional genetic modification may be a knock-out or reduced activity/transcription/translation levels via editing in coding sequences, promoters, introns, regulatory regions, for example in the RB1 or TP53 genes, or in another example, the genetic mutation may be a knock-in or increased activity/transcription/translation levels via editing in coding sequences, promoters, introns, regulatory regions, for example for the RAS gene;

In one example, the animal cell has a genetic modification in *RB1*.
In one example, the animal cell has a genetic modification in *TP53*.
In one example, the animal cell has a genetic modification in a *RAS* gene.
In one example, the animal cell has a genetic modification in *RB1* and in *TP53*.
In one example, the animal cell has a genetic modification in *RB1* and in a *RAS* gene.
In one example, the animal cell has a genetic modification in *TP53* and in a *RAS* gene.
In one example, the animal cell has a genetic modification in *RB1*, *TP53* and in a *RAS* gene.
In one example, the *RAS* gene is *HRAS, NRAS, or KRAS*.
In one example, the *RAS* gene is *HRAS.*

According to a second aspect of the invention, there is provided a method of producing cultivated meat or a cultured meat product comprising culturing the animal cell as described herein.

According to a third aspect of the invention, there is provided a method of producing the cultured animal cell as described herein.

According to an example, there is provided a method of producing the modified animal cell as described herein.

In one example, the animal is selected from a pig, bovine, poultry, sheep, goat, Equidae, Camelidae, fish, crustaceans or mollusc.

In one example, the animal cell is a somatic cell.

In one example, the animal cell is selected from one of the following cell types: myoblast, fibroblast, myofibroblast, adipose derived stem cell, epithelial cell, mesenchymal stem cell, satellite cell, iPSC, or hepatocyte.

In one example, the modification is introduced using targeted genome modification.

According to a fourth aspect of the invention, there is provided a cultivated or cultured animal tissue or a cultivated or cultured meat product comprising a modified cell as described herein.

In one example, the culture is a suspension culture.

According to a fifth aspect of the invention, there is provided use of the modified cultured animal cell as described herein for cellular agriculture.

According to an example, there is provided use of the modified animal cell as described herein for cellular agriculture.

According to a sixth aspect of the invention, there is provided a method for producing an immortalised animal cell line comprising the method as described herein wherein the immortalised cell line comprises a NF2 gene modification.

In one example, the modification is a loss of function modification or leads to reduction in function..

In one example, the loss of function modification is a knock-out of the *NF2* gene.

According to a seventh aspect of the invention, there is provided a guide RNA targeting the sequence of SEQ ID NO. 5, or SEQ ID NO. 8, or SEQ ID NO. 9, or SEQ ID NO. 10, or SEQ ID NO. 28, or SEQ ID NO. 29, or SEQ ID NO. 30 individually or in combination.

According to an eighth aspect of the invention, there is provided a kit comprising at least one of the guide RNA as described herein.

Figures and tables

The invention is further described in the following non-limiting figures.

Figure 1 is a graph that shows the doubling times in suspension conditions. Doubling time assessment of control cell pools (transfected with Cas9 only, no sgRNA) and NF2 knock-out cell pool after transfer from adherent into suspension growth conditions. Data points come from triplicate Erlenmeyer flasks and measured over 4 growth passages.

Figure 2 is a graph that shows the collective data from all 4 passages of the doubling time assessment from Figure 1

Figure 3 is a graph that shows the NF2 gene editing efficiencies. Assessment of NF2 knock-out efficiencies in porcine myoblast cell pools transfected with Cas9 protein and a sgRNA targeting the NF2 gene. At two time points post transfection and after a 4 passage suspension growth study in triplicate flasks, DNA was isolated from cell pools and knock-out efficiencies were determined by PCR amplification of the NF2 gene followed by Sanger Sequencing of the PCR amplicon and analysis of the sequencing results using the ICE analysis tool (ice.synthego.com).

Figure 4 shows functional knock-out of NF2 gene coding for the Merlin protein preserves mesenchymal cell phenotype and enhances adipogenic differentiation potential in immortalised myoblast cell lines. (a) Flow cytometry density plots for cell surface markers CD29 (mesenchymal), CD56 (myoblast), CD90 (mesenchymal), CD29 CD56+/+ (muscle stem cell), CD31 (endothelial) and CD45 (immune lineage) evidencing loss of CD56 and CD90 expression following adaptation of NF2 (+/+) cells to suspension from adherent culture conditions, which is retained following functional knockout of the NF2 gene. (b-g) Quantification of flow cytometry data for corresponding surface markers in NF2 (+/+, purple bars) and (-/-, green bars) suspension cell lines. (h) Fluorescence micrographs of NF2 (+/+) and NF2 (-/-) cell lines labelled for lipid marker BODIPY (green) and nuclear DNA (DAPI, blue) in suspension shows enhanced