Written Component about article 'Loss of Neogenin1 in human colorectal carcinoma cells causes a partial EMT and wound-healing response'

Q1: Describe the most important methodology used in the manuscript and how it was used to address a biological question

The most important methodology used in this research is the combined usage of immunofluorescence and confocal microscopy.

Immunofluorescence is a commonly used immunostaining method. It uses the specific-binding property of antibodies to their antigens to combine fluorescent dyes with targeted biomolecules in cells. Hence, it allows the visualization of the quantity and the distribution of targeted molecules throughout the sample cells.

Confocal microscopy is a special type of fluorescence microscopy. It can generate immunostaining images of higher resolution and wider field compared with normal fluorescence microscopy.

In this research, cells in the control group and Neo1-depleted group are incubated with appropriate primary antibodies that target the interested proteins and then use corresponding second antibodies that combined with fluorescent dyes 'Alexa fluor'.

In this way, under confocal microscopy, we can indirectly know the expression level, morphology and localization of these proteins of interest by observing the fluorescence intensity and localization. By comparing the image of fluorescent-dyed protein in control cells and Neo1-depleted cells, we can know how the loss of Neo1 changed the expression of these proteins.

For example, 'Figure5a' in this assigned article (as shown in **Fig.1**) shows the distribution of microtubules (MTs) by immunostaining alpha-tubulin. Then by comparing the confocal microscopy images, we can see the distribution of MTs has changed in Neo1-depleted cells, suggesting the loss of Neo1 causes the morphology of these cells more 'mesenchymal'.

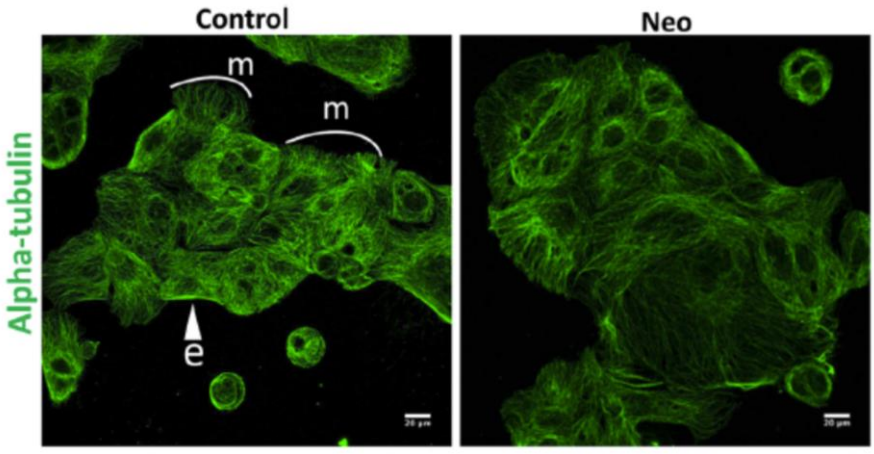

Fig1. 'Figure5a' in the assigned article. Showing control and Neo1-depleted cells stained for alpha-tubulin.

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Q2: Describe in detail a bioinformatics- or experiment-based approach that could be used to take the research further.

Targeted DNA sequencing technology and relevant variation calling tools

To take this research further, we can apply targeted DNA sequencing technologies on different types of tumour samples, to explore the potential correlation of the mutation of *Neo-1* and carcinogenesis and identify the types of *NEO-1* mutation. The correlation might be helpful with precision medicine and gene therapy in cancer treatment.

Targeted DNA sequencing is to specifically sequence selected regions of the genome or genes of interest, instead of the whole genome [1]. Compared with whole-genome sequencing, targeted sequencing can achieve higher sequencing depth, and hence higher specificity and sensitivity. This enables more accurate variant identification, especially for those rare and novel variants. Also, targeted sequencing is much more time-saving and cost-effective when sequencing a large number of samples.

The process of targeted sequencing includes the design of probes, capture of targets, library construction, and finally pair-end sequencing [2]. After that, we need to combine multiple bioinformatics software to go through read normalization, alignment to the reference genome, variant calling, etc.

In this research, we can collect tumour samples from different types of cancers and in different stages (maybe focus more on tumours in the metastasis stage). Then we can perform targeted sequencing towards *NEO-1* as well as the other relevant genes involved such as *CEACAM1, ANXA1*, and then call variants. If a significant proportion of samples are found having variants within these mentioned genes, then we can indicate the correlation between these variations and the corresponding type and stage of cancers.

Word count: 249

Reference

1. Harismendy O, Ng PC, Strausberg RL, Wang X, Stockwell TB, Beeson KY, Schork NJ, Murray SS, Topol EJ, Levy S, Frazer KA: **Evaluation of next generation sequencing platforms for population targeted sequencing studies.** *Genome Biology* 2009, **10:**R32.
2. O'Roak BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG, Carvill G, Kumar A, Lee C, Ankenman K: **Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders.** *Science* 2012, **338:**1619-1622.