ECM X: A methodological approach to tracing cell lineage in human tissues.

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INTRODUCTION: Methods are badly needed that can identify stem cell niches and allow lineage tracing from stem cell progeny in human tissues. Studying stem cell dynamics and clonal populations in human tissue presents a number of unique challenges because of the inherent experimental limitations involved, and to date there has been no reliable means to do this. In this review we describe techniques for lineage tracing in human epithelial tissues, taking as our exemplars the intestine, liver, pancreas and skin. We believe that the method has general applicability, and is limited only by the availability of fresh-frozen human

METHODS: Fresh frozen blocks of human tissue of normal histological appearance were used. mtDNA-encoded cytochrome *c* oxidase (COX)-deficient patches were identified. Cells from COX-positive and deficient areas were cut by laser microdissection. Cell digestion and DNA extraction was performed by overnight incubation in a DNA extraction kit (Picopure™, Arcturus, CA) at 65°C. The extracted DNA was used to sequence the entire mitochondrial genome from microdissected areas. A two-round amplification method was followed, whereby the first round consisted of amplifying 9 fragments spanning the entire genome, and the second round consisted of 36 M13-tailed primer pairs to amplify overlapping segments of the first-round products. Sequencing was performed using the BigDye terminator cycle sequencing method on an ABI Prism Genetic Analyzer and compared to the revised Cambridge reference sequence using sequence alignment software of the European Molecular Biology Open Software Suite (EMBOSS; www.ebi.ac.uk/emboss/align/). COX expression could also be examined by immunohistochemistry (IHC) on routine formalin-fixed tissues.

RESULTS: We can show that human stem cells and their progeny contain non-pathogenic mutations in their mitochondrial DNA, including mutations in the cytochrome *c* oxidase (*COX*) gene - a component of complex IV of the respiratory chain, that are relatively common. IHC in human liver identified patches of COX-deficient hepatocytes located in close proximity or in direct contact with the portal tract region (*Fig*).

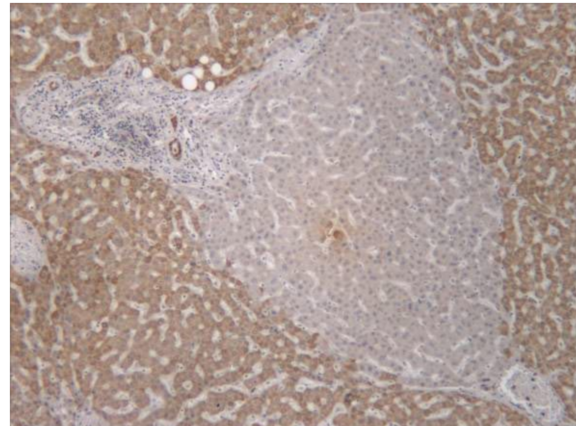

Fig.1: A COX-deficient patch stretching from the portal area (top left) to the hepatic vein (bottom right).

mtDNA sequencing is not possible on this fixed material, however in frozen sections identical mutations were present in each cell of a particular patch, thus establishing their clonal origin. Clonally-derived patches were also seen in the pancreas, and in gastric glands and intestinal crypts (tissue units with multiple stem cells) we find ribbons of COX-deficient cells, also clonal in origin, allowing us to map the fate of the progeny of individual stem cells.

DISCUSSION & CONCLUSIONS: We have described a method for cell lineage tracing especially, but not exclusively, in epithelial tissues, where organised architecture is the rule. Limitations of the method are that such mutations take some time to be established, and thus observations are so far confined to individuals over the age of 40. There is also the limitation that snap-frozen tissue is necessary for definitive analysis by double-enzyme histochemistry and mtDNA mutation analysis.