

LATE OSTEOBLAST/EARLY OSTEOCYTE-LIKE CELL LINE FOR VISUALIZING COLLAGEN ASSEMBLY IN LIVING CELLS

Immortal cell lines representing the late osteoblast/early osteocyte phenotype have been generated that stably express a collagen-GFP or collagen-mCherry fusion protein to fluorescently label type I collagen fibrils either red or green. These novel cell lines allow visualization of collagen fibril assembly in living cells over time, which is not possible with existing technologies. The only other approaches that have been used for monitoring collagen assembly in living cells include using fluorescently labeled antibodies to collagen or a fluorescently labeled recombinant bacterial protein that binds to collagen. These have the disadvantage over our new invention that they may potentially interfere with the protein function and that they only label a population of fibrils at one point in time, which can then be followed (i.e. they do not necessarily label new collagen as it is synthesized). The specificity of the bacterial binding protein for type I collagen as opposed to other collagens is unclear and neither of these probes can be used to follow intracellular steps in the collagen assembly pathway, as they do not cross the cell membrane. Therefore our collagen-GFP and collagen-mCherry probes represent a significant improvement over existing technologies.

POTENTIAL AREAS OF APPLICATIONS:

- Potential commercial applications of this invention include using these cell lines to screen for drugs that enhance collagen assembly and could therefore have potential as bone anabolic treatments for diseases such as osteoporosis. The cells can also be used to screen for drugs that inhibit collagen assembly and therefore have potential as treatments to prevent fibrosis, etc. The cells also have many potential uses in developing approaches for tissue engineering of bone tissues. For example, the cells can be seeded onto scaffolds and the assembly of collagen can be monitored in real time in the living cultures. Mineral deposition on collagen can be monitored simultaneously using vital dyes for calcium deposition. These cell lines may also have commercial application for looking at mechanisms of tissue destruction, such as degradation of matrix proteins by proteases, such as occurs during inflammation.

PATENT STATUS: Provisional filed

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