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Human artificial chromosomes for future biomedicine

Comment on: Liskovykh M, et al. Stable maintenance of de novo assembled human artificial chromosomes in embryonic stem cells and their differentiated progeny in mice. Cell Cycle 2015; http://dx.doi.org/10.1080/15384101.2015.1014151

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The engineered mammalian artificial chromosome (MAC) emerged as a new technology in 1991.[2,3] The idea of a non-integrating, safe, autonomously replicating, mitotically and meiotically stable[4] chromosomal vector with an almost unlimited gene-carrying capacity was immediately catching fire. Many groups worldwide commenced to develop their own methods to produce such a promising gene carrier (recently reviewed in[5,6]). Applications of MACs are widespread. MACs are excellent material for basic research, since they facilitate studies about chromosome structure and stability, chromatin assembly, centromere and kinetochore organization, telomere maintenance, chromosomal movements, replication and segregation. We are able to load MACs with multiple genes[7] and then conduct research on full biochemical and signal transduction pathways by using only one genetic vector. It is possible to engineer these pathways either to be active or inactive by including inducible promoters and/or gene silencing methods. Unlike other genetic vectors (viruses, plasmids, transposons, PACs, BACs, YACs), MACs are able to harbor the full genomic sequences of genes with all the introns and regulatory elements that are needed for their proper spatial and temporal expression.[5,6] MACs encoding synthetic or natural genetic networks can be used to program pluripotent and multipotent stem cells to follow specific lineage pathways and produce specific cell types. These studies are prone to reveal how certain cell types are formed from stem and progenitor cells. Moreover, these differentiated cell types could be suitable for cell therapy applications. MACs are able to express marker genes to facilitate the separation of differentiated cells from undifferentiated ones. It is also possible to build suicide genes into MACs. This could make cell and gene therapy safer. Genetic mutations (monogenic and polygenic also) can be repaired in patient's cells with MACs carrying and expressing the wild type version of the mutant gene before cell therapy. This could lead to personalized cell and gene therapy applications. Recent discoveries of stem/progenitor cells preferentially home to tumor sites offer the possibility for tumor therapy with MACs armed with an arsenal of transgenes, including a drug-prodrug suicide system, cytokines and antibodies. Recently, MAC technology developed further. Previous MACs were mostly derived from existing natural chromosomes or fragments thereof. A new technology allowed the manufacturing of MACs, in this particular case a human artificial chromosome (HAC), in a completely synthetic way.[5] This bottom-up approach of HAC generation was initially developed in human HT1080 cells. Human cells were transfected with either natural high-order repeat or synthetic α-satellite (alphoid) DNA cloned into a circular BAC vector or into a linear YAC vector carrying telomeric sequences. In all cases, HAC formation was achieved by 20- to 30-fold multimerization of the input DNA. Since then several groups have reported the successful generation of de novo constructed HACs and the size of those HACs ranges from 1 to 10 Mb. These HACs have been shown to be mitotically stable both in human and rodent cells. In this issue of Cell Cycle, Alexey Tomilin and his group took one enormous step further with HAC technology.[1] For the first time, they successfully delivered an alphoid^{tetO}-HAC into mouse embryonic stem (ES) cells. They also demonstrated the stable maintenance of this artificial chromosome throughout differentiation of HAC-carrier ES cells into somatic cell types of adult mice. It is an important milestone in the field of artificial chromosome technology. These synthetic alphoid^{tetO}-HACs might become valuable tools to investigate rodent models of human diseases and to screen for therapeutic molecules for a possible cure. Germline transmission and stability of a 60Mb rodent artificial chromosome was previously reported.[4] We expect to see such an achievement with these alphoid^{tetO}-HACs in the near future. Stable inheritance of these HACs in established mouse model strains would facilitate research on human disease models including genetic as well as epigenetic disorders, infectious diseases and cancers. These invaluable model mice would be available worldwide for basic research and also for pharmaceutical drug testing.

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