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NEWS

The reproducibility challenge

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Webinar: The bone field discusses the reproducibility of results in preclinical studies.

In order that new knowledge can be used to improve human health, scientists and the public need to be able to trust the results of scientific inquiry. The biomedical research enterprise stands and falls on this premise. However, in recent years there have emerged deep concerns[1] about whether results from preclinical studies in animal models can be adequately reproduced by other researchers and thereby confirmed.

The bone field is not immune to such worries. In an article in the *Journal of Bone and Mineral Research*[2] and in this webinar, Stavros Manolagas of the University of Arkansas Medical Sciences and Henry Kronenberg of Harvard Medical School guide viewers through the major issues as they pertain to bone research.

The biggest challenge is the variable nature of animal models. Bone researchers frequently use mice so they can study bones in a living system. However, mouse bones are not human bones; 'they aren't even rat bones', says Kronenberg. For example, many groups have shown that bisphosphonates and parathyroid hormone (PTH) have additive effects on bone mass in rodents,[3] but the same effects are generally not seen in human studies.[4] Researchers need to make sure they are careful in extrapolating findings in mice to human biology and disease.

The strain of mouse can also affect the viability of a study. Kronenberg's work with mice lacking the PTH/PTHrP receptor showed that in one common mouse model, the C57BL6 strain, almost all mice died early in gestation, probably because of abnormal cardiac development, making the study of bone development impossible.[5] His team was only able to reliably define the bone phenotype when he used another, genetically distinct, mouse strain.

Genetically modifying mouse models brings another set of challenges.[2] Gene knockout experiments, for example, can involve inactivating anything from little segments of DNA to large stretches, with profound effects on the mouse. Knocking out a whole gene may affect other nearby genes; knocking out a gene in the germ line may affect the whole of an animal's development. It is always best to knock out as little of a gene as you need to disable it, but knowing how much can be fraught.

Other genetic manipulations are more subtle—for example, inserting promoters to drive expression of a particular gene. However, even these need to be used with care. Researchers cannot assume that the transgene will behave as the normal gene does, or that the promoter ends up in exactly the right place in the genome.

Likewise, the Cre-lox system, which enables genes to be knocked out only in specific tissue types, might create a scenario that is unlike normal development. For instance, Cre promoters may be active in bone in adulthood, but in a developing mouse they may show activity in different locations. Finally, there are even more sophisticated versions of Cre-lox where the Cre promoter remains inert until activated by a drug such as tamoxifen or doxycycline. However, these drugs both have their own effects on bone. Doxycycline binds tightly to bone mineral, for example, so takes longer to be removed from the body and hence to relax its effect on the Cre. It is important to understand these effects and prevent them from influencing the results of a study.

All new research methods have their own off-target effects and unanticipated consequences that can affect whether other researchers are able to replicate your results. New experimental techniques need to be monitored carefully and interpreted cautiously, with alternative explanations considered alongside the favoured one. Researchers should use creativity and care in equal measure. The whole bone research community is responsible for the trustworthiness of its results.

For more details on reproducibility in bone research, watch the accompanying webinar http://www.nature.com/bonekey/webinars/index.html?key=webinar34.

Edited by LJ Suva

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