O camundongo mutante obeso ob/ob, que não produz leptina (Lep-Ob$^{-/-}$), apresenta quadro de resistência a insulina e inflamação do tecido adiposo, típico da Diabetes Mellitus Tipo 2, sendo um ótimo modelo de estudo para essas doenças. Como os animais Lep-Ob$^{-/-}$ são inférteis, a manutenção das linhagens requer a identificação dos animais Lep-Ob$^{+/-}$ para reprodução. Como a obtenção de DNA normalmente é invasiva, desenvolvemos um método não invasivo baseado em esfregaços orais para a obtenção de DNA: foram coletadas células da mucosa oral de 18 camundongos (2 Lep-Ob$^{+/+}$, 1 Lep-Ob$^{-/-}$ e 15 animais desconhecidos) com espátulas de polietileno. As células foram ressuspensas em 200 μL de solução TES (10 mM Tris-HCl pH 7,6; 1mM EDTA; 0,6% SDS) e agitadas por 30 s com posterior adição de 10 μL de uma solução 10 mg/mL de Proteinase K e incubação por 2 h a 42°C. As proteínas foram precipitadas com 15 μL de solução de NaCl 6M, seguida de centrifugação (15.000g/4min, 22°C) e coleta do sobrenadante. 450 μL de etanol absoluto foram adicionados aos sobrenadantes, e em seguida foram centrifugados como anteriormente. O sobrenadante foi descartado e, por duas vezes, o precipitado foi ressuspenso em 300 uL de etanol 70% e re-centrifugado, com descarte do sobrenadante. Os precipitados foram ressuspensos em 15 uL de Tris-HCl 10 mM pH 8,0 e foi realizada a quantificação do DNA (média de 14,5 ±7,6 μg/μL). Foi realizada PCR (50 ciclos) em tempo real com iniciadores para a região mutada do gene da leptina e 50 μg do DNA extraído, gerando produtos com 715 ± 137 μg/μL de DNA. 900 μg desse produto foram enzimaticamente cortados com 5U de enzimas de restrição DdeI (2 h a 37°C), e os fragmentos foram separados através de eletroforese de gel de agarose a 4% com brometo de etídio. Animais Lep-Ob$^{+/+}$ apresentaram uma banda de 155pb, animais mutantes Lep-Ob$^{-/-}$ apresentaram 2 bandas de 55 e 100pb, e animais heterozigotos apresentaram as 3 bandas (155, 100 e 55pb). Assim, é possível a obtenção de DNA e genotipagem dos animais através dessa técnica simples, relativamente barata e que não causa traumas nem riscos aos animais.

A rapid non-invasive method of genotyping leptin-deficient mutant mice by oral swab sampling and restriction digestion

Obesity is a widespread epidemics intimately related to the development of type 2 diabetes mellitus (T2DM). Hence, in vivo models of obesity are of value for the study of T2DM. The spontaneous mutant obese ob/ob mouse, which does not produce leptin (Lep-Ob$^{-/-}$), presents an attractive model of insulin resistance and adipose tissue inflammation, typical of T2DM. However, if Lep-Ob$^{-/-}$ animals are easily distinguished from the normal mice because of the evident obesity presented already in the early weeks after birth, heterozygous Lep-Ob$^{+/--}$ are phenotypically indistinguishable from normal Lep-Ob$^{+/+}$ animals. Moreover, Lep-Ob$^{-/-}$ mice are infertile so that laboratory matings should be performed by using only heterozygous animals, which implicates genotyping. Since the excision of a piece of mouse tail or venipuncture for obtaining DNA samples are difficult to be employed in newborns, we developed a simple non-invasive method based in oral epithelial cells for genotyping the animals.

By using oral swabs or polyethylene microspatulas, the oral mucosa of 18 mice (2 heterozygous controls, 1 Lep-Ob$^{-/-}$ control and 15 unknown animals) was scraped twice. The cells were resuspended in 500-µL microtubes containing 200 µL of TES solution (10mM Tris-HCl pH 7.6; 1mM EDTA; 0,6% SDS) and vortexed for 30 s. Then, 10 µL of a 10 mg/mL Proteinase K solution was added to each tube, which were incubated for 120 min at 42°C. Afterwards, proteins were precipitated with 15 µL of 6 M NaCl and samples were centrifuged at 15,000 x *g* (4 min, room temperature). Then, supernatant fractions were transferred to a fresh 1.5-mL microtube and mixed with 450 µL of absolute ethanol and then centrifuged at 15,000 x *g* (4 min, room temperature) again. The pellets containing DNA were resuspended in 300 µL of 70% (v/v) ethanol solution, and the tubes were centrifuged again under the same conditions. After supernatant discard, the pellets were dissolved in 15 µL of 10 mM Tris-HCl pH 8.0; and DNA contents were spectrophotometerically determined (average yield: 14.5 ±7.6 µg/µL). Aliquots of 50 µg DNA were run for real-time PCR amplification (50 cycles) with Stratagene Brilliant® II SYBR® Green qPCR Master Mix and primers (FW: TGT CCA AGA TGG ACC AGA CTC; RV: ACT GGT CTG AGG CAG GGA GCA) yielding typically 715 ± 137 µg/µL DNA. Then, 900 ng of PCR product were enzymatically cut with 5 units of *DdeI* restriction enzyme (2 h, 37°C) and DNA fragments were resolved in 4% agarose. Whereas digests from wild mice presented a characteristic single 155 bp band, mutant Lep-Ob$^{-/-}$ showed 2 bands (55 and 100 bp) and heterozygous samples showed 3 bands (55, 100 and 155 bp) allowing for the rapid characterization of any littermate.

With this simple, not time-consuming technique, mouse DNA may be obtained and analyzed in less than 6 h allowing the genotyping of leptin mutant mouse newborns without the need of venipunctures and the risk of infections or mutilations. The method is safe, relatively inexpensive, time-sparing and reliable.

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