School of Medicine, PO Box 208059, New Haven, CT 06520

E-mail: brittany.craiglow@yale.edu

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A technique for more precise distinction between catagen and telogen human hair follicles ex vivo

To the Editor: Identifying human anagen hair follicles (HFs) ex vivo is readily accomplished by stereomicroscopic analysis. However, to reliably distinguish other hair cycle stages, namely late catagen and telogen, by stereomicroscopic analysis alone is difficult, and the gold standard remains histologic analysis, which obviously precludes their use for ex vivo culture.[1,2] In this study, we sought to determine whether methylene blue, a staining that can be applied to living cells,[3] helps to distinguish late catagen from telogen HFs intravitally for subsequent organ culture, thus expanding translational preclinical research into these poorly investigated, but clinically important, human hair cycle stages.

Using follicular unit hair transplantation methodology (by grouping follicular units on the basis of the number of HFs they contain),[4] we recorded the number of anagen, catagen, and telogen follicles found in 800 follicular units from 8 white male patients (100 follicular units/patient) undergoing a standardized follicular unit extraction hair transplant procedure, with informed patient consent. Because anagen VI follicles are easily identifiable,[1] only those follicular units that contained catagen or telogen HFs were further microdissected, photographed, immersed ~5 minutes in 0.02% methylene blue saline solution, fixed, and evaluated.

Intravital methylene blue staining enhanced anatomic HF structures on light microscopy (Fig 1, *A-C*) and permitted correct hair cycle stage classification using accepted, well-defined morphologic criteria,[2] such as the identification of a prominent epithelial strand (Fig 1, *A*), a key feature of late catagen HFs that is absent in telogen HFs. Correct hair cycle stage classification by this method was confirmed by Ki67 and TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labeling) immunofluorescence microscopy (Fig 1, *D*).

Importantly, methylene blue staining enabled correct identification of the hair stage of 95.63% of cases, compared with 72.02% of nonmethylene blue–stained HFs. Thus, this simple, economical, and fast technique constitutes a significant methodologic advance in human hair research, since it facilitates ex vivo research on human catagen and telogen HFs without having to resort to histology.

Our analyses revealed a higher percentage of catagen than telogen HFs in all patients (89% anagen, 6.7% catagen, and 3.6% telogen). This data support the previous proposal that the percentage of scalp telogen HFs has been overestimated[2] and suggest we should question the accepted standard percentages (80%-89% anagen, 10%-20% telogen, and 1%-5% catagen) in the literature, which were based on transversal histologic sections[5] and phototrichograms, neither of which can definitively distinguish between late catagen and telogen HFs. Although, in our study, the HFs were from patients with androgenetic alopecia (AGA) and the ratio of anagen:catagen:telogen might differ in comparison with individuals without AGA, we believe that our data are unlikely to reflect sampling bias, as HFs were harvested from occipital scalp, generally unaffected by AGA. We propose that hair stage distribution in healthy human scalp needs a more systematic re-evaluation, including comparative studies with histologic sections. This is important when assessing candidate hair growth–modulating agents, considering minor shifts in the percentage of telogen or catagen HFs can result in major changes in the degree of visible effluvium.

Irene Hernandez, PhD,[a] Majid Alam, PhD,[a,b,c] Christopher Platt, PhD,[d] Jonathan Hardman, PhD,[d] Eleanor Smart, MSc,[d] Enrique Poblet, MD,[e] Marta Bertolini, PhD,[c,f] Ralf Paus, MD,[d,g] and Francisco Jimenez, MD[a,b,b]

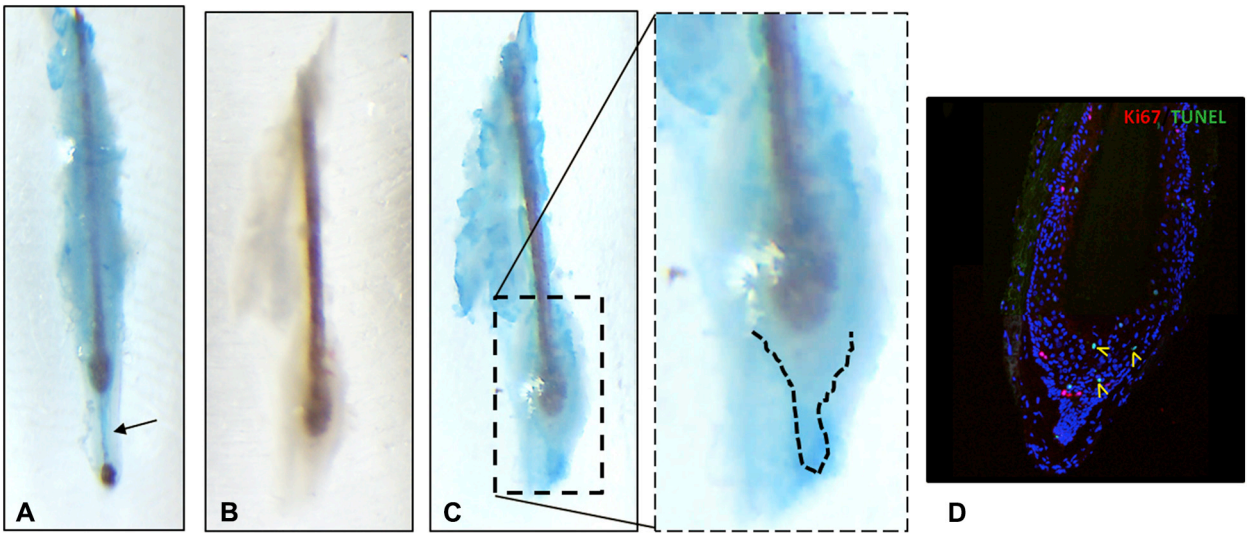

Fig 1. Macroscopic analysis of hair follicles isolated from follicular units is more definitive after methylene blue staining. **A**, Hair follicle in late catagen stage, with the epithelial strand (*arrow*) clearly visible after methylene blue (0.02%) staining. **B**, Nonstained hair follicle that cannot be clearly identified under the stereomicroscope as either late catagen or telogen, but after methylene blue staining (**C**), the small remaining epithelial strand (*dotted-line*) allows us to identify it as late catagen. **D**, Ki67 staining and TUNEL confirms this strand is a late catagen hair follicle, with the presence of several apoptotic, TUNEL positive cells (*arrowheads*). *TUNEL*, Terminal deoxynucleotidyl transferase dUTP nick-end labeling.

From the Mediteknia Clinic and Hair Laboratory, Las Palmas, Spain[a]; Universidad Fernando Pessoa Canarias, Las Palmas, Spain[b]; Monasterium Laboratory, Münster, Germany[c]; Centre for Dermatology Research, University of Manchester, Manchester, United Kingdom[d]; Department of Pathology, University General Hospital and Murcia University, Murcia, Spain[e]; Department of Dermatology, University of Münster, Münster, Germany[f]; Department of Dermatology, Miller School of Medicine, University of Miami, Miami, Florida[g]; and Medical Pathology Group, IUIBS, Universidad de Las Palmas Gran Canaria, Las Palmas, Spain[b]

Drs Alam and Platt contributed to this work equally.

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Reprint requests: Francisco Jimenez, MD, Mediteknia Clinic, Av Alcalde Ramirez Bethencourt 20, Las Palmas de Gran Canaria, 35004, Canary Islands, Spain

E-mail: fjimenez@mediteknia.com

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Whole-exome sequencing reveals differences between nail apparatus melanoma and acral melanoma

To the Editor: Nail apparatus melanoma (NAM) is considered a subtype of acral melanoma (AM). A study in Korea reported that 50% of melanomas in Koreans were AM.[1] AM and cutaneous melanoma are considered distinct on the basis of genetic evidence; AM typically has a low single-nucleotide variant (SNV) burden, high numbers of focal amplifications, and frequent *TERT* aberrations compared