Materials and Methods

Sebocyte cultures

Human SZ95 sebocyte cultures [16] were enzymatically removed from the culture flasks and centrifuged in sterile Eppendorf vials. Subsequently, the supernatants were removed and the agglutinated sebocytes were transferred to clean glass slides, where they were observed and photographed using a fluorescence microscope (FM-YG100; Soochow FZM Optical Tech, China) under white light and long-wave UV light, respectively. The sebocyte fluorescence spectrum excited by UV (peaks at 390 nm) was documented by a microfluorospectrometer (FX2000; Idea Optics, Shanghai, China) connected to the microscope.

Inter- and intracellular sebaceous lipids were detected by staining with Nile Red, as previously described [17]. In short, distilled water was added to agglutinated sebocytes from a SZ95 sebocyte suspension, then a small drop of the suspension was transferred to a polylysine-coated glass slide, which was transiently heated by a spirit lamp for several times until complete evaporation of the liquid. The cells were then stained with Nile Red in dimethyl sulfoxide for 5 min and were observed using the fluorescence microscope with blue light (peak at 440 nm) for excitement.

Sebaceous glands

UVRF of sebaceous glands isolated from fresh human skin samples was examined. The samples were donated by 15 patients undergoing skin tumor surgery at the Shanghai Skin Disease Hospital. The healthy security margin of the excised skin was used in this study. The samples were trimmed into small cubes immediately after excision, clamped in a stainless steel vise, and frozen at –20℃ for about 30 min (online suppl. Fig. S1a). The frozen tissue together with the vise were quickly observed under a stereomicroscope. The vertical plane of the skin sample was cut by a sterile scalpel to obtain slices that contained intact pilosebaceous units (online suppl. Fig. S1b). The sebaceous glands in the hand-made slices were observed under white and UV light, and the spectrum of sebaceous glands was documented by the microfluorospectrometer.

The samples were then embedded in paraffin, sliced, and stained with hematoxylin-eosin to confirm the identity of the anatomical structures. To avoid possible interference of non-sebaceous gland tissue, we also examined the fluorescence of sebaceous glands isolated by dispase I according to Xia et al. [17], then confirmed the presence of sebum within the sebaceous glands by Nile Red stain.

Sebum

Sebum from 50 sebaceous glands isolated from the patients was extracted in order to evaluate UVRF emission by sebum. The sebaceous glands were isolated from skin slices using microsurgical instruments under a stereomicroscope, then were collectively immersed in diethyl ether and agitated for 2 h at 18℃ in a dark environment. The solution was transferred to a clean biochemistry vial, heated at 50℃ for 2 h to devolatilize diethyl ether, and then examined under UV light. Sebum was collected directly from fresh sebaceous glands rather than from the skin surface in order to avoid possible modifications of sebum components through the influence of the follicular environment.

Microorganisms

UVRF-positive follicular casts were sampled from 5 healthy volunteers and their fluorescence spectra documented. Subsequently, microorganisms from the casts were isolated by anaerobic culture on blood agar plates, since UVRF has been shown to originate from the deeper, anaerobic part of the follicles [11]. Microbial colonies were observed under long-wave UV light and the red fluorescent colonies, if any, were purified by subculture. The UVRF spectrum of the colonies was documented by a microfluorospectrometer and the identity of the colonies was confirmed by 16S rRNA sequencing.

The UVRF spectra of the isolated bacteria colonies and the follicular casts were compared. Porphyrins were extracted by ethyl acetate from one isolated *P. acnes* strain according to a previous study [18], and the UV fluorescence spectrum of the extraction was compared with those of the bacteria colony and the follicular casts.

The colonies of other follicle microorganisms were also examined under UV light to detect whether they emitted UVRF. These microorganisms were isolated in own previous studies as well as in the current one.