SUPPLEMENTARY INFORMATION

FROM:

Stratum-Specific Expression of Human Transferrin Receptor Increases Iron in Mouse Epidermis

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Figure S1 (jpeg 58K)

(**a**) Transgene expression in transfected mouse keratinocytes. Cultured cells were labeled with ^{35}S. Extracted protein was immunoprecipitated with antibody B3/25 and human transferrin receptor identified on SDS-PAGE by autoradiography. HK – positive control of human keratinocytes. MK – negative control of BALB/c keratinocytes. MK+K14hTFR – BALB/c keratinocytes transfected with the hTfR construct and labeled 72 hours later. (**b**) Synthesis of human transferrin receptor in keratinocytes isolated from K14-hTfR transgenic mice. Cell lines were established from six littermates, labeled with ^{35}S, and analyzed for human transferrin receptor synthesis as in (**a**). PCR of genomic DNA using primers specific for the human transferrin receptor distinguished transgenic (pcr+) from wild-type (pcr-) littermates. The labeled band at approximately 92 kDa, the expected size of the human transferrin receptor (hTfR), was seen in only the pcr+ cells. A non-specifically precipitating protein was seen in all lanes, and serves as an internal loading control. (**c** and **d**) Expression of hTfR is limited to lower layers of epidermis in transgenic mice. Immunohistochemistry, using Neomarker antibody to human transferrin receptor, was done on tail skin from a K14-hTfR transgenic mouse. Expression is most intense in the basal layer of the epidermis and sebaceous glands and the outer root sheath of hair follicles of transgenic animals (**c**), but is absent from the upper layers of epidermis and totally absent from skin of non-transgenic littermates (**d**). (**e**) mRNA for the hTfR transgene is found in the epidermis only in K14-hTfR transgenic mice. Reverse transcriptase-PCR was performed on RNA extracted from epidermis and liver of littermates. The 18 0bp pcr-amplified cDNA product was identified in epidermis of two animals that were positive for the transgene in genomic DNA (+) and absent from two animals that were negative for the transgene in genomic DNA (-). RNA from liver (L), which should not express protein from a K14 promoter, did not yield amplified product. In separate reactions, the 525 bp band, using primers for glyceraldehyde-3-phosphate dehydrogenase, verified the presence of amplifiable cDNA. Positive pcr control=genomic DNA(D); negative pcr control (C)=water.

hTfR1: 5'-CTGCTATGGGACTATTGCTGTG-3'.

hTfR2: 5'-CCGACAACTTTCTCTTCAGGTC-3'.

GAPDH1: 5'CTCCTTGGAGGCCATGTAGGCCATG.

GAPDH2: 5'GCAACTGCTTAGGGGGGGTGGCCAG.

Figure S2 (jpeg 60K)

(**a**) Transgene expression in transfected mouse keratinocytes. Cultured cells were labeled with ^{35}S. Extracted protein was immunoprecipitated with antibody B3/25 and human transferrin receptor identified on SDS-PAGE by autoradiography. HK – positive control of human keratinocytes. MK – negative control of BALB/c keratinocytes. MK+Inv-hTFR – BALB/c keratinocytes transfected with

the hTfR construct and labeled 72 hours later. (**b**) Synthesis of human transferrin receptor in keratinocytes isolated from Inv-hTfR transgenic mice is regulated by calcium. Cell lines were established from two transgenic animals, cultured for 2 days in either low calcium (lo) or high calcium (hi), labeled with ^{35}S and analyzed for human transferrin receptor synthesis (hTfR) as in (**a**). The labeled band at approximately 92 kDa, the expected size of the human transferrin receptor, was faint in cells grown in low calcium but strong in cells grown in high calcium. (**c** and **d**) Expression of hTfR is limited to the upper layers of the epidermis in transgenic mice. Immunohistochemistry, using Neomarker antibody to human transferrin receptor, was done on sections of tail skin from an Inv-hTfR transgenic mouse. In transgenic animals (**c**), expression is most intense above the basal layer of the epidermis and the inner root sheath of hairs follicles. Staining was absent from sebaceous glands and the outer root sheath of hair follicles and totally absent from skin of non-transgenic littermates (**d**). (**e**) mRNA for the hTfR transgene is found in the epidermis only in Inv-hTfR transgenic mice. Reverse transcriptase-PCR was performed on RNA extracted from the epidermis and liver of littermates. The 180 bp pcr-amplified cDNA product was identified in the epidermis of two animals that were positive for the transgene in genomic DNA (+) and absent from two animals that were negative for the transgene in genomic DNA (-). RNA from liver (L), which should not express protein from an Inv promoter, did not yield amplified product. In separate reactions, the 525 bp band, using primers for glyceraldehyde-3-phosphate dehydrogenase, verified the presence of amplifiable cDNA. Positive pcr control=genomic DNA(D); (see Figure Supplementary 1e for primers used).

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