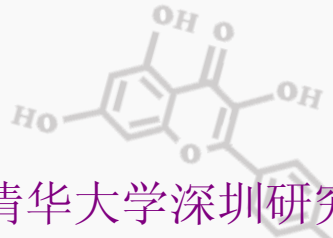
清華大學
Tsinghua University

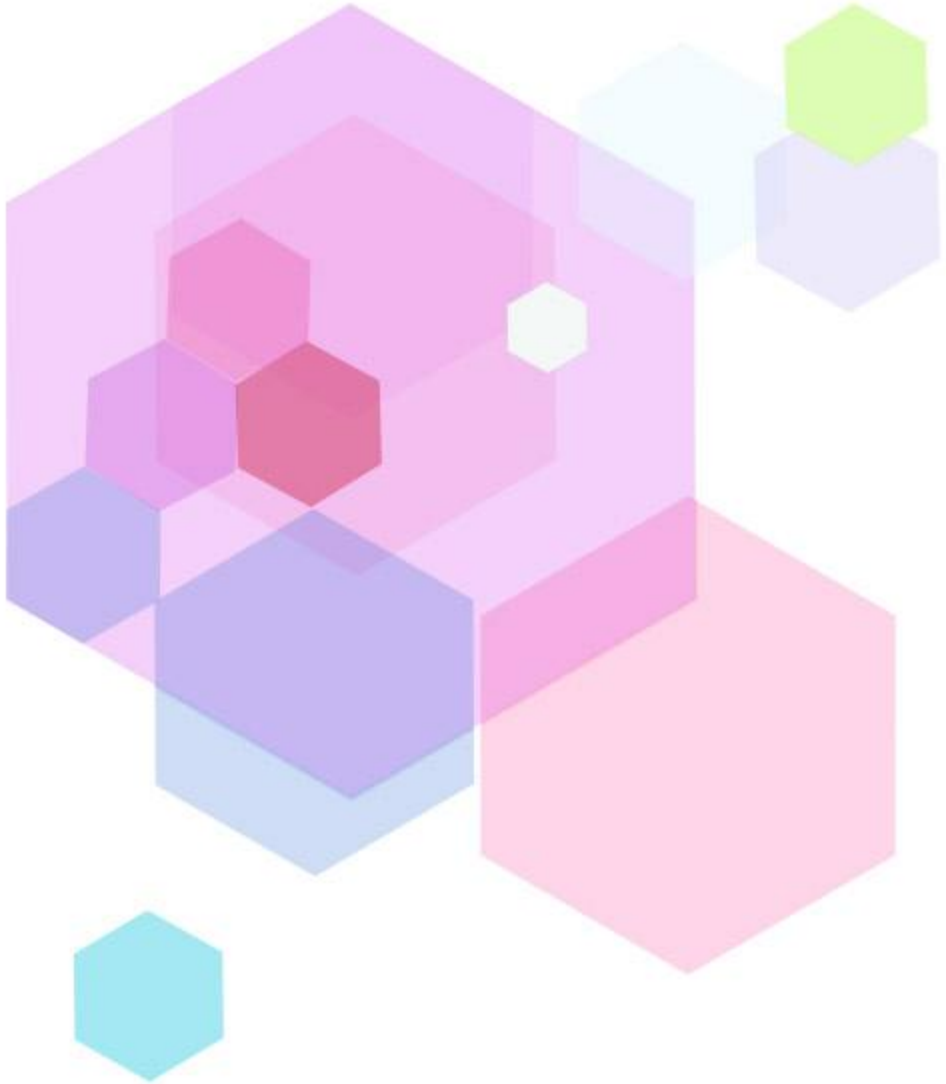
黄酮类化合物依赖皮肤黑色素介导的UVB辐射损伤保护

The protection of flavonoid-mediated melanocyte against UVR



史小军 PhD

清华大学深圳研究生院
生物技术与创新药物研究联合实验室



- 背景
- 我们的研究
- 探讨与展望

背景

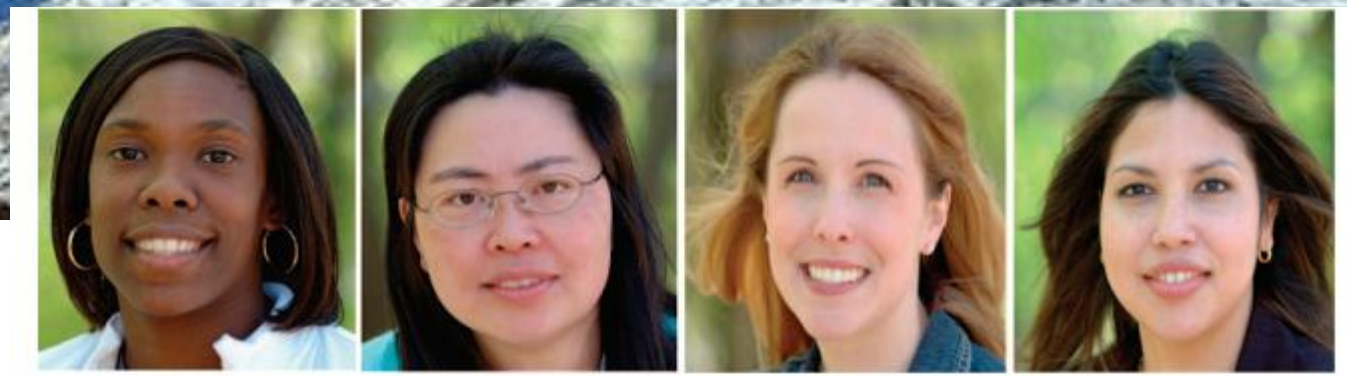
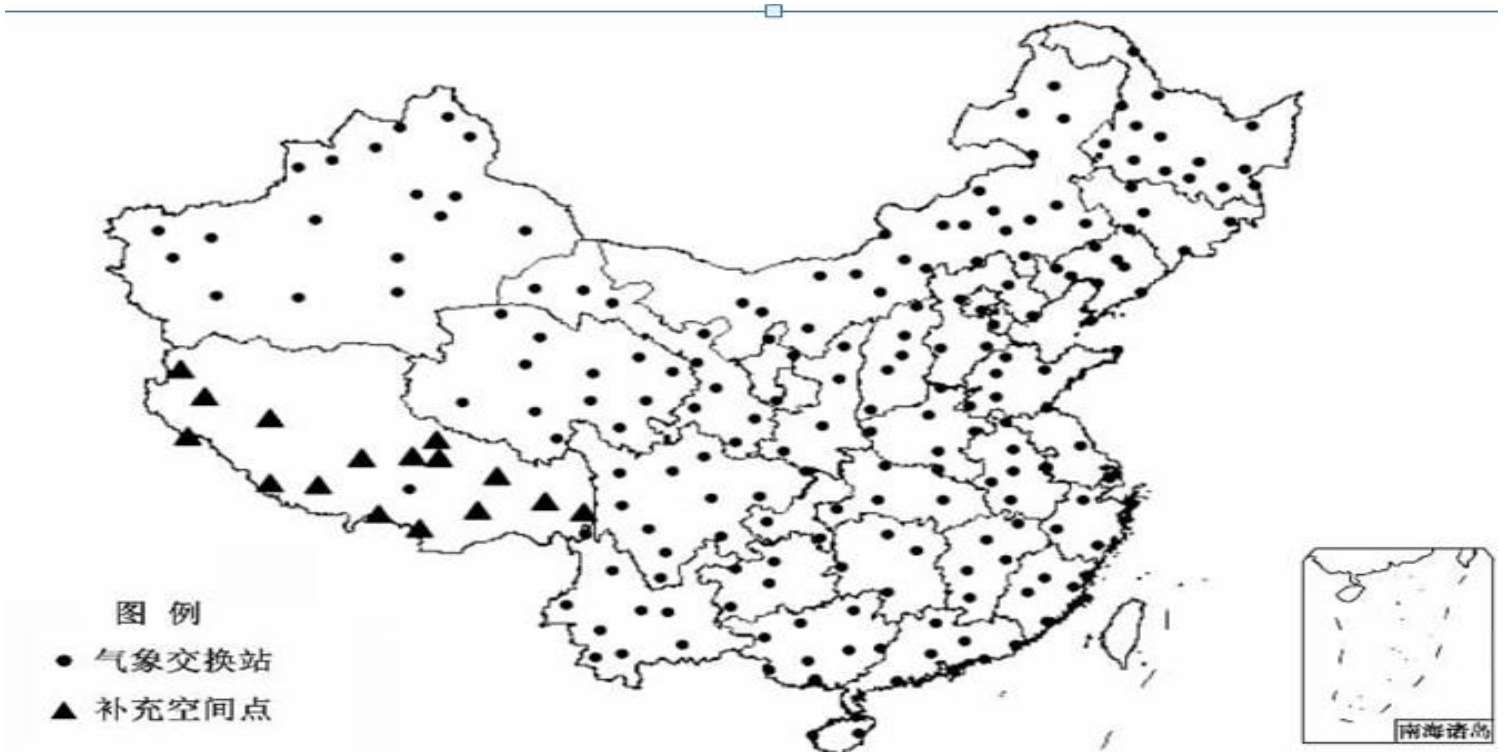


Figure 3. Human pigmentation—the main skin types: African-American, Asian, Caucasian, and Hispanic (left to right).

辐射

低频电磁

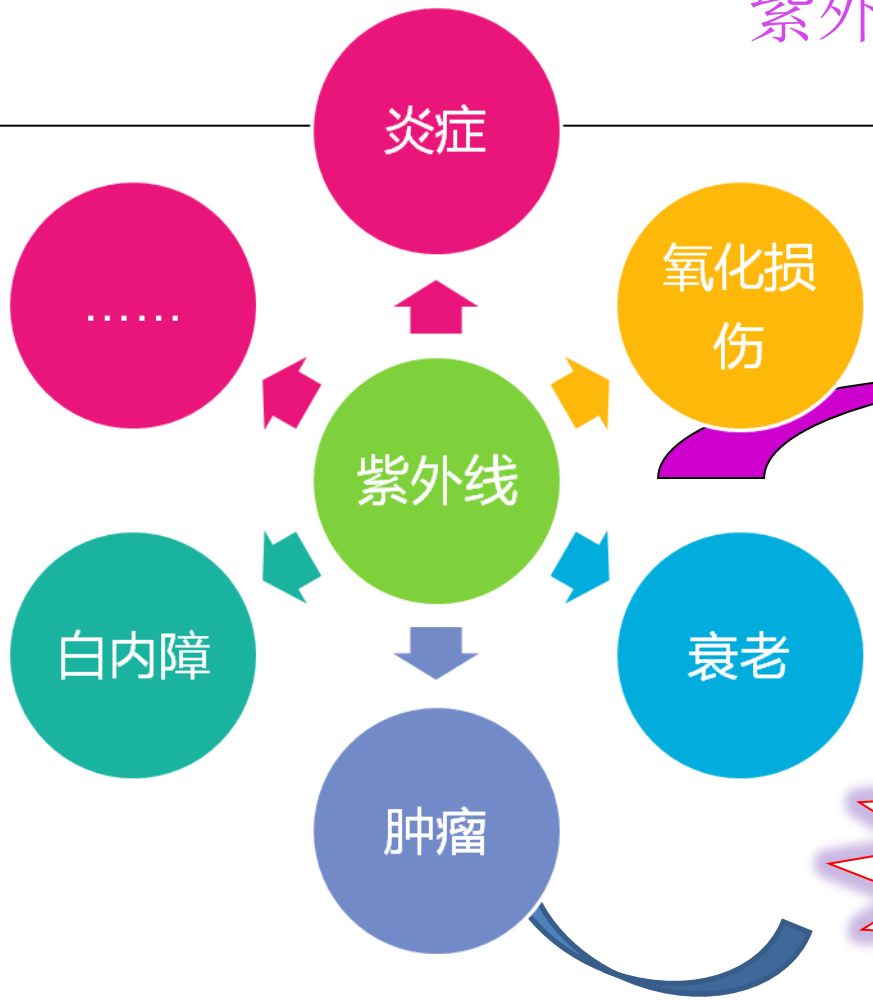
过量紫外线



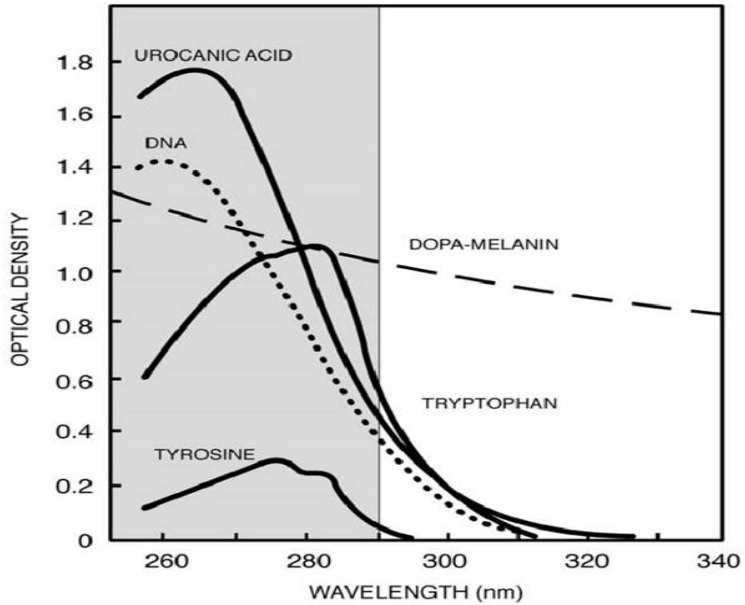
陆面生物有效紫外线辐射强度模拟空间点阵

到达陆面的生物有效紫外线辐射对**人体健康的影响取决于日辐射总量和日辐射峰值.**

紫外线、 低频辐射



皮肤屏障功能

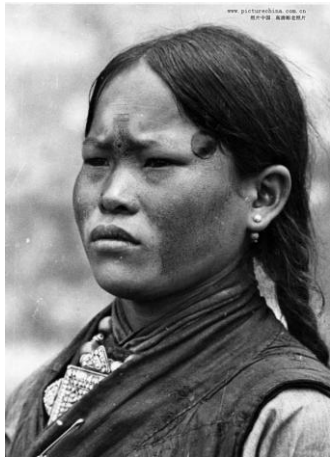


UVR absorption spectra of molecules important to UV-induced health effects DOPA-melanin-synthetic model of eumelanin

皮肤屏障
功能

黑色素瘤的发病率正在上升。在美国在1935年的黑色素瘤的终生风险是在1/1500人，1960年是/1600,2000年，1/75人

Environmental Health 2012, 11(Suppl 1):S12

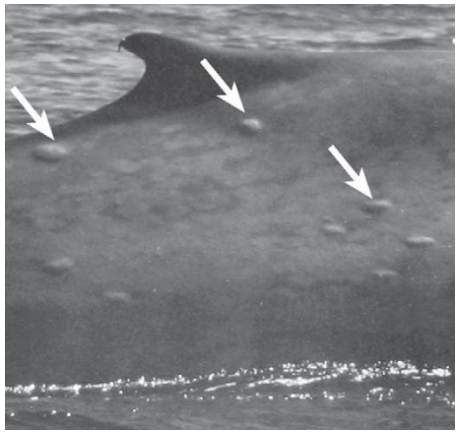


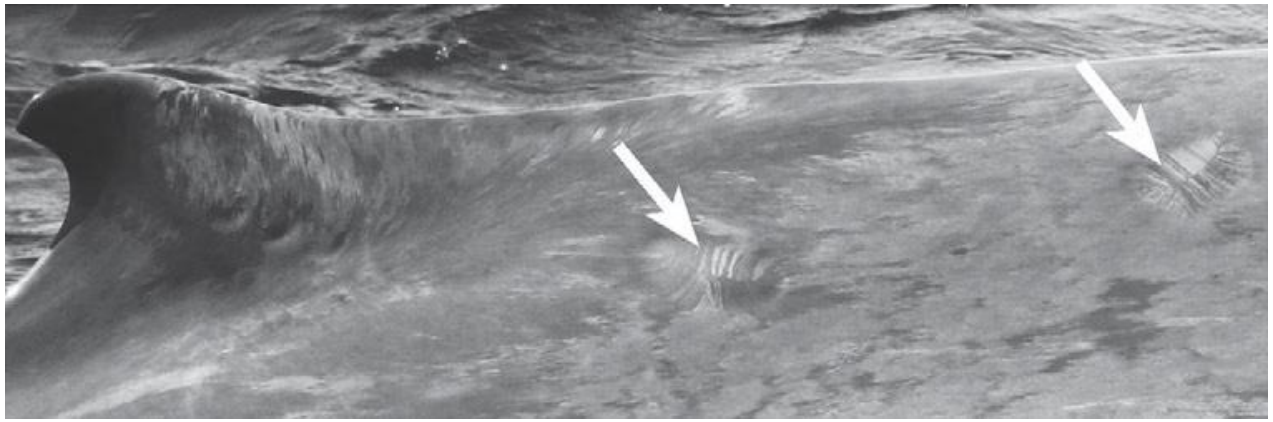
UV is culprit for the development of skin cancer. Chronic UV -actinic keratosis, squamous鳞状 、basal cell cancer, intermittent and intense UV exposure melanoma黑色素瘤

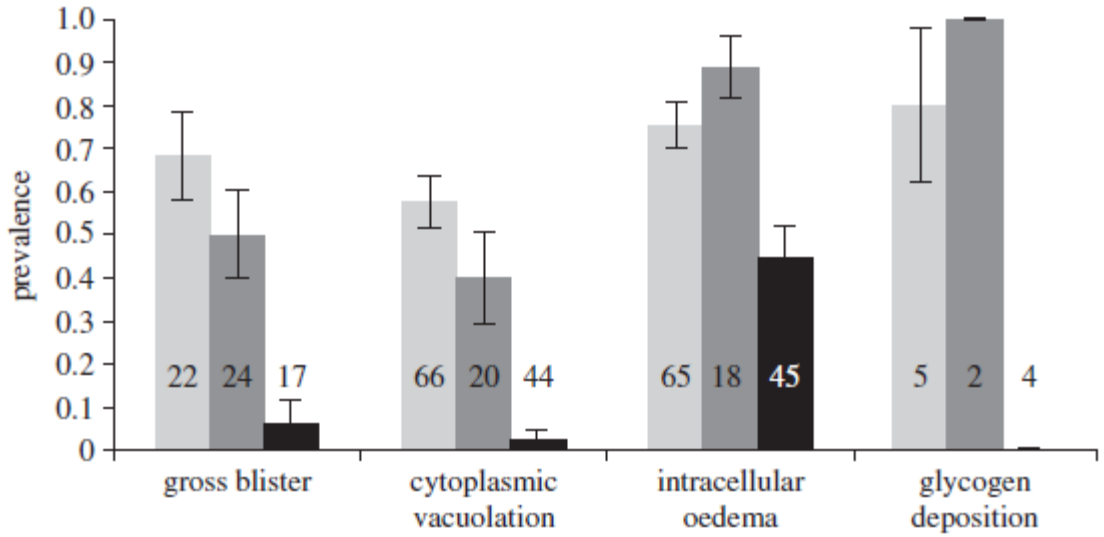
UV Accelerates the photoaging wrinkling, dryness, telangiectasia(毛细血管扩张),sagging, and pigmentation.

Photoprotection: a Review of the Current and Future Technologies

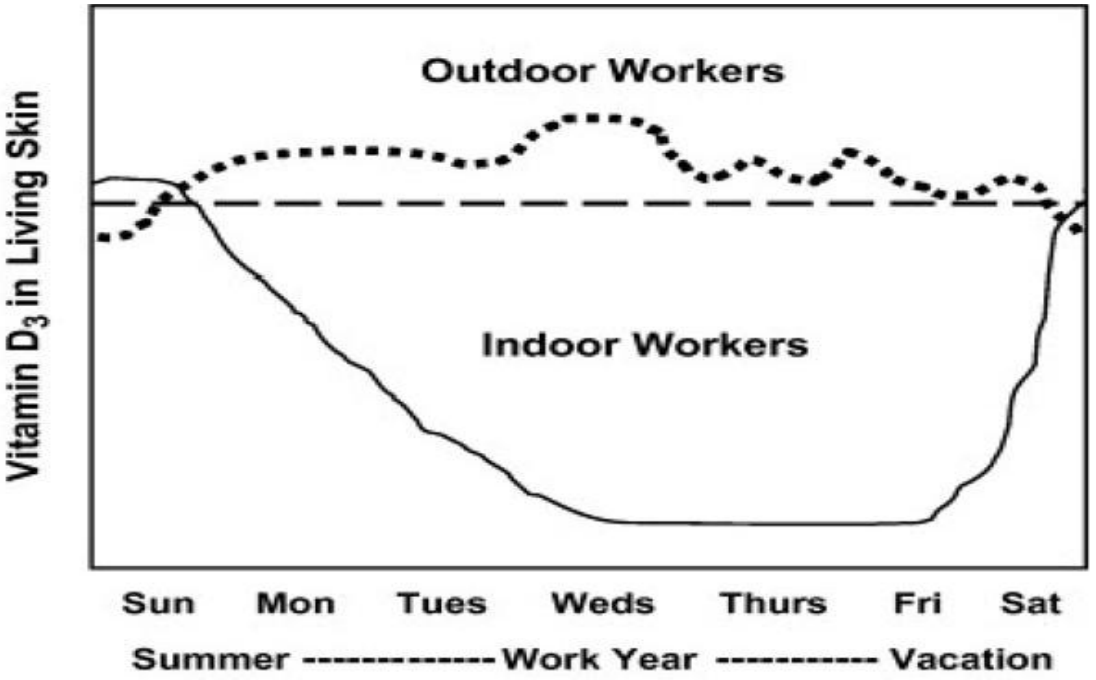
Dermatologic Therapy, Vol. 23, 2010, 31–47





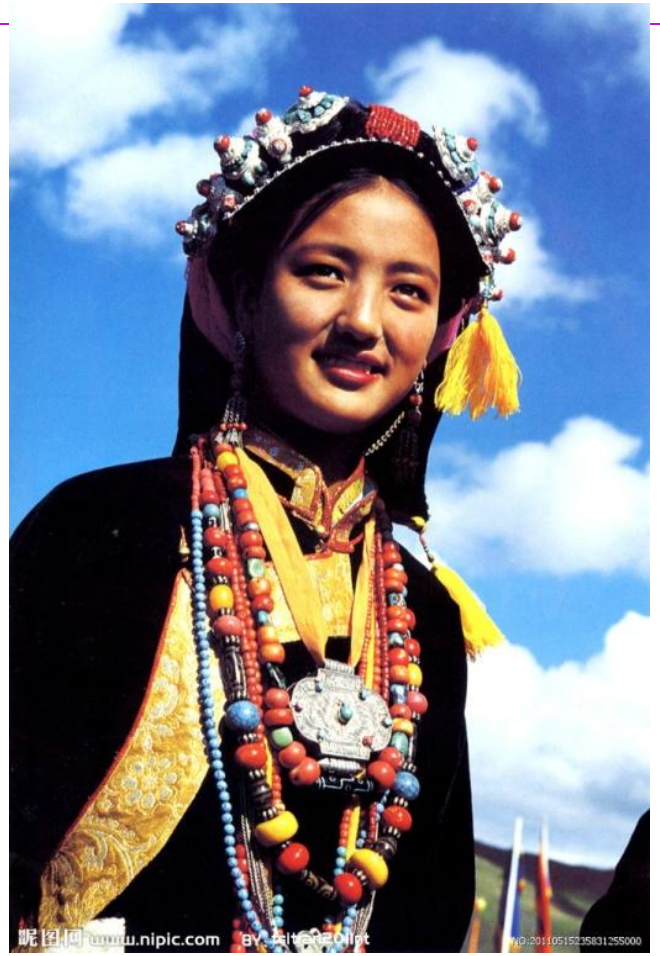


Acute sun damage and photoprotective responses in whales
Proc. R. Soc. B (2011)



Cutaneous vitamin D3 The cutaneous vitamin D3 “roller coaster” that indoor workers experience during the workweek and workyear compared to outdoor workers. (Godar D.E.,2009).

- 红斑反应
- 皮肤色素沉着
- 皮肤光老化
- 皮肤光敏反应
- 皮肤免疫抑制
- 皮肤肿瘤



UVB辐射引起的皮肤过黑化（Hyperpigmentation）

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1 课题背景

过量UVB

皮肤过黑化

颜色不均
斑点
痣、痦
黑色素瘤
黑色素细胞癌
……

*Reference: The integumentary system: anatomy, physiology and fnction of skin

UVB辐射引起的皮肤过黑化（Hyperpigmentation）

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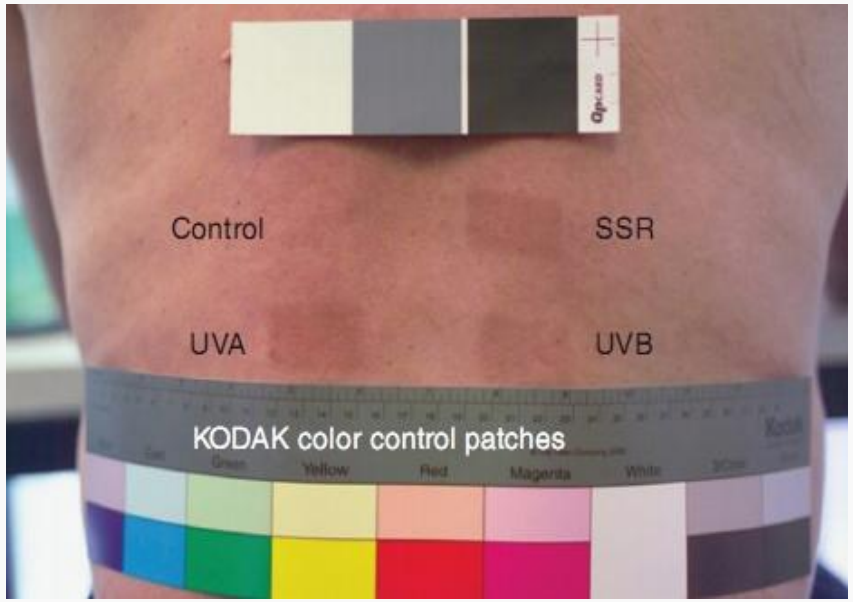
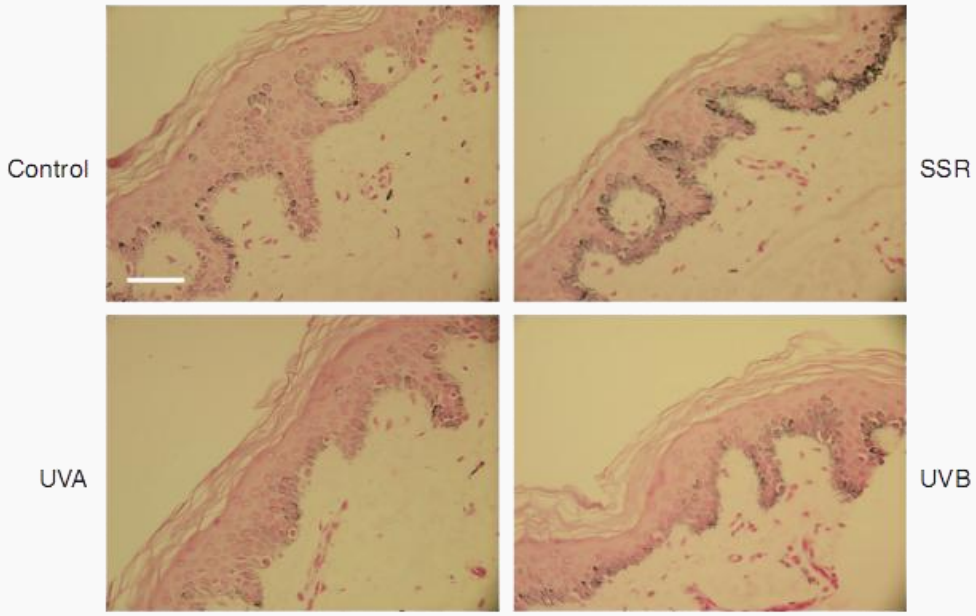
1 课题背景





	Control	SSR	UVA	UVB
Melanin	28.39 ± 4.61	39.34 ± 3.98*	27.69 ± 4.53	38.86 ± 5.46*

"UVB (with or without UVA) significantly increased the melanin content (39 or 37%, respectively) in all six subjects, but UVA alone had no significant effect compared with the unirradiated control."

*Reference: Regulation of Human Skin Pigmentation in situ by Repetitive UV Exposure: Molecular Characterization of Responses to UVA and/or UVB

UVB辐射引起的皮肤过黑化（Hyperpigmentation）

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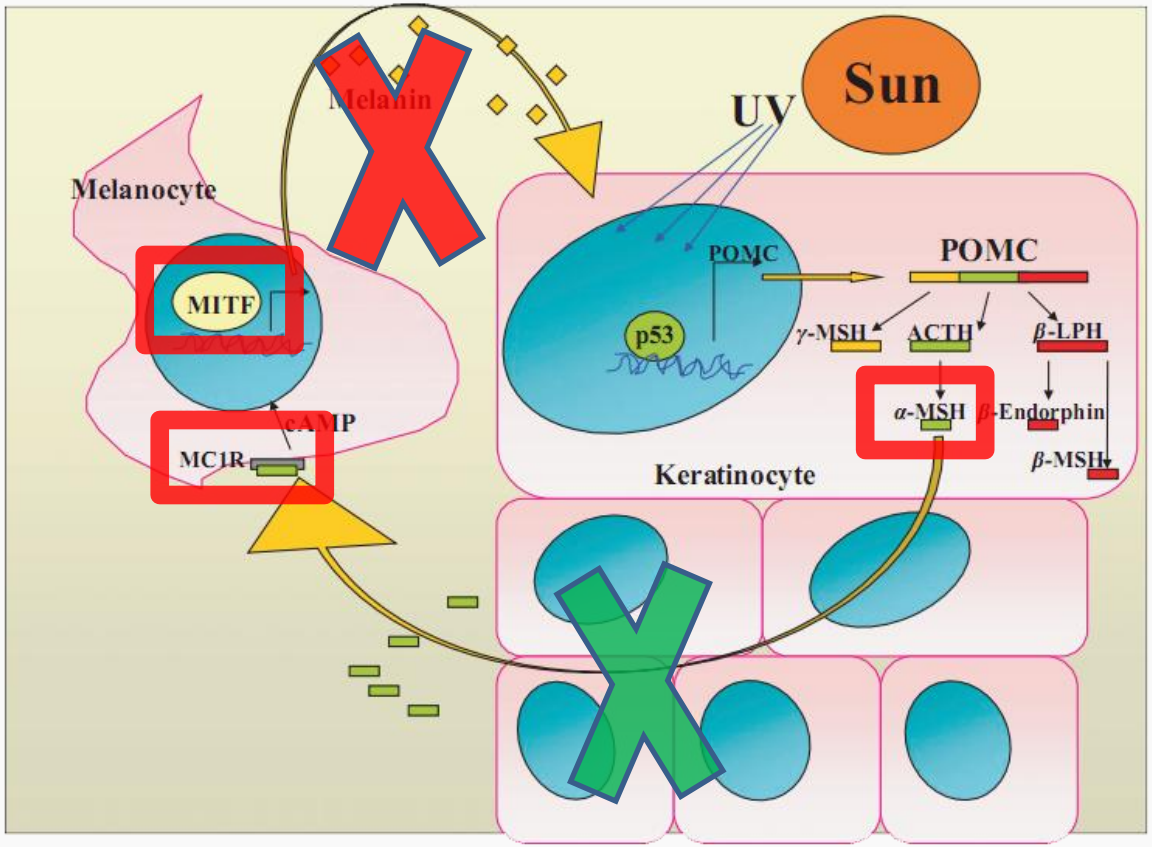
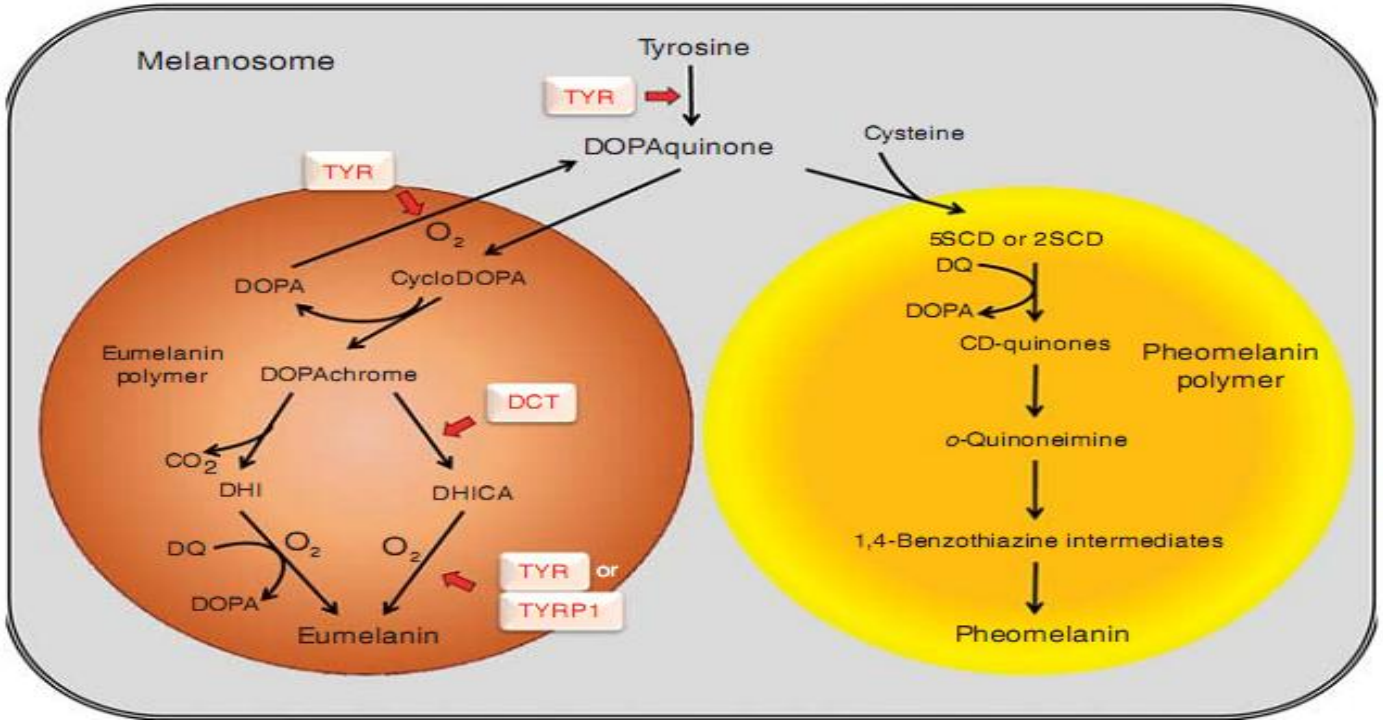
1 课题背景



Key Factors:

- Epidermal（表皮）:
  Alpha-MSH
- Melanocyte（黑色素细胞）:
  - MITF Tyrosinase
  - Tyrosinase related protein 1
  - Tyrosinase related protein 2
  - ……

*Reference: Lighting a path to pigmentation: mechanisms of MITF induction by UV



✓DCT即TRP2

✓TYRP1即TRP1

ＴＲＰ－１和ＴＲＰ－２是黑色素细胞和恶性黑色素瘤细胞中含量最丰富的一种糖蛋白。ＴＲＰ－１在黑色素合成的下游途径中起重要作用。ＴＲＰ－１突变可引起肤色变浅，ＴＲＰ－１能与磷酸化的酪氨酸酶形成复合体，从而可能对酪氨酸酶的激活和稳定起作用，抑制ＴＲＰ－１的表达会引起黑色素体的结构异常。ＴＲＰ－２可与酪氨酸酶和ＴＲＰ－１形成复合物，在黑色素合成过程中，ＴＲＰ－２将多巴色素催化为羧化物衍生物５，６－二羟吲哚－２－羧酸（ＤＨＩＣＡ）

Antioxidant compounds	Source	End point	Study Human	Animal	Reference
Flavonoids					
Genistein	Soy, red clover, ginkgo biloba	Erythema	+	+	(90,91)
		Photoaging		+	(90)
		Immunosuppression		+	(92)
		Photocarcinogenesis		+	(90)
Equol	Metabolite of daidzein	Erythema, inflammation		+	(91,92)
		Photoaging		+	(93)
		Immunosuppression		+	(92)
		Photocarcinogenesis		+	(94)
Apigenin	Fruits and leafy vegetables, tea, wine	Photocarcinogenesis		+	(95)
Daidzein	Soybeans and plant, red clover	Erythema		+	(91)
Silymarin/silibinin	Milk thistle/ bioactive component of silymarin	Immunosuppression		+	(96)
		Photocarcinogenesis		+	(97,98)
Caffeic, ferulic acids	Vegetables, olive, olive oil	Erythema	+		(99)
Polypodium leucotomos extract	Tropical fern plant *Polypodium leucotomos*	Erythema	+		(100)
		Photoaging		+	(101)
		Photocarcinogenesis		+	(101)
Pycnogenol	Extract from the bark of the maritime pine tree	Inflammation		+	(102)
		Immunosuppression		+	(102)
		Photocarcinogenesis		+	(102)
Resveratrol	Skin and seeds of grapes, nuts, fruits, red wine	Edema		+	(103)
		Photocarcinogenesis		+	(104)
Vitamin C (L-ascorbic acid)	Most fruits and vegetables	Erythema	+	+	(105)[a], (106)
		Immunosuppression		+	(107)
		Photoaging		+	(108)
		Photocarcinogenesis		+	(108)
Vitamin E (α tocopherol)	Plant oils	Erythema	+		(109)
		Immunosuppression		+	(110)
		Photoaging		+	(108)
		Photocarcinogenesis		+	(111)
Green tea polyphenols (epigallocatechin-3-gallate), (epicatechin-3-gallate), (epigallocatechin), (epicatechin)	Polyphenolic fractions isolated from tea	Erythema	+	+	(112,113,114)
		Immunosuppression		+	(115,116)
		Photocarcinogenesis		+	(117–119)
		Photoaging		+	(114)

黄酮类化合物-Quercetin

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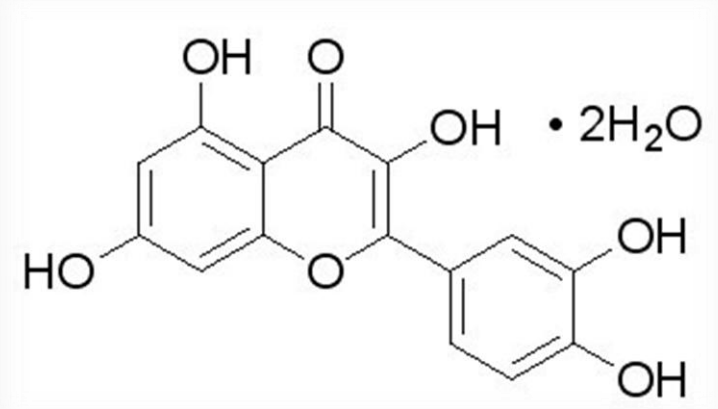
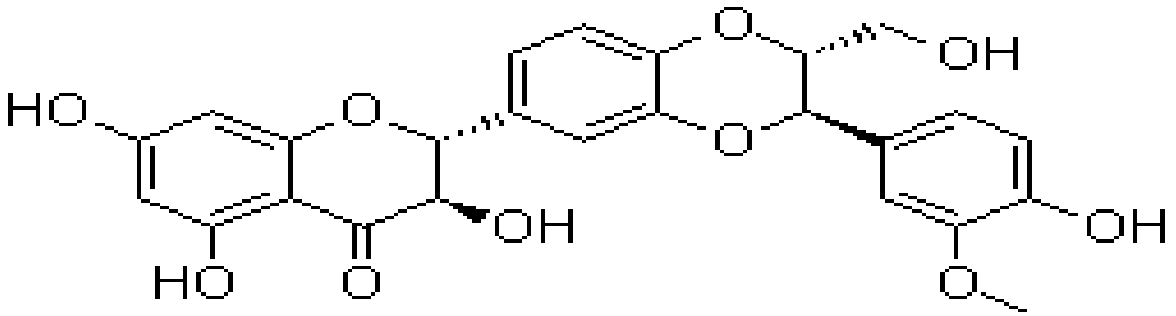
1 课题背景



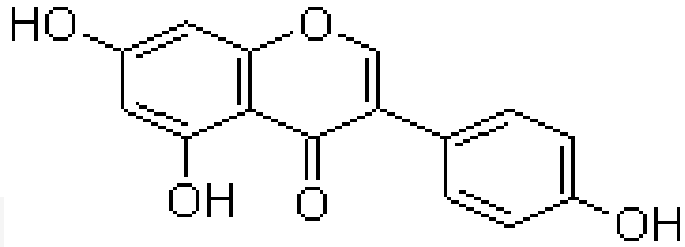
天然活性物质

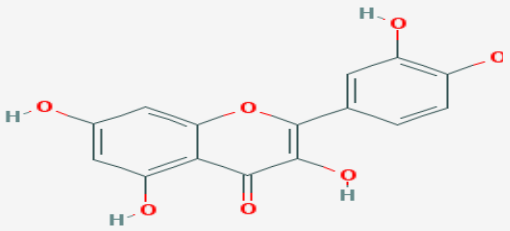
- 黄酮醇
- 抗氧化Anti-oxidation
- 抗炎症Anti-inflamtion
- 抗肿瘤Anti-tumor

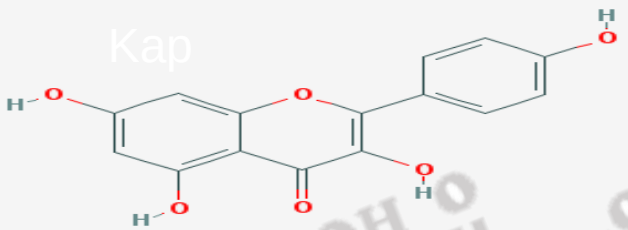
*Reference: USDA database for the flavonoid content of selected foods.

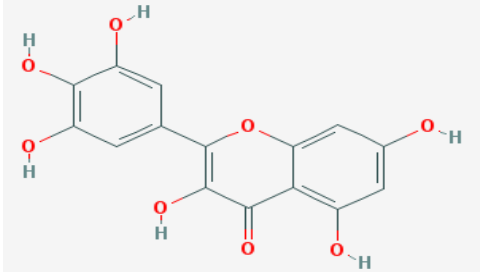


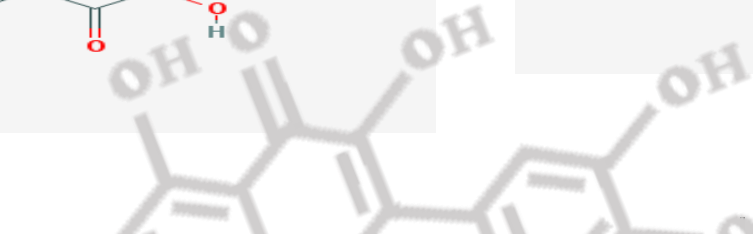
染料木素

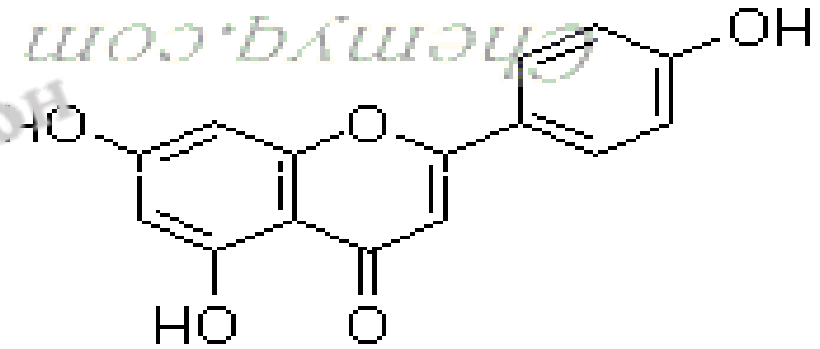


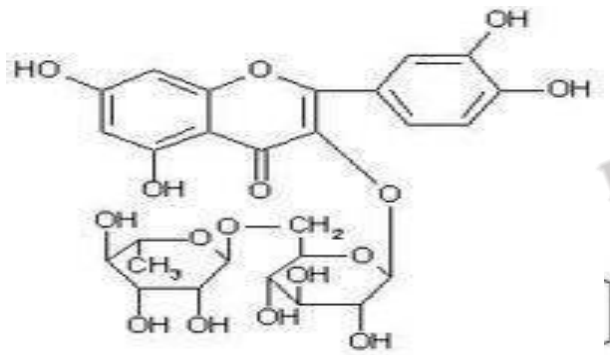


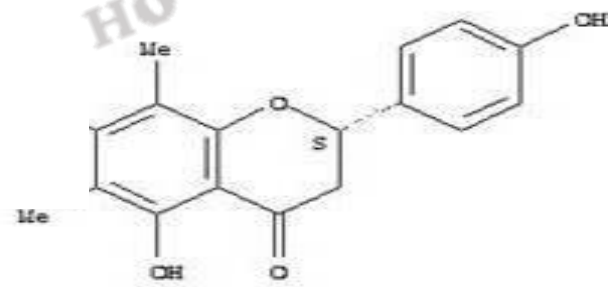


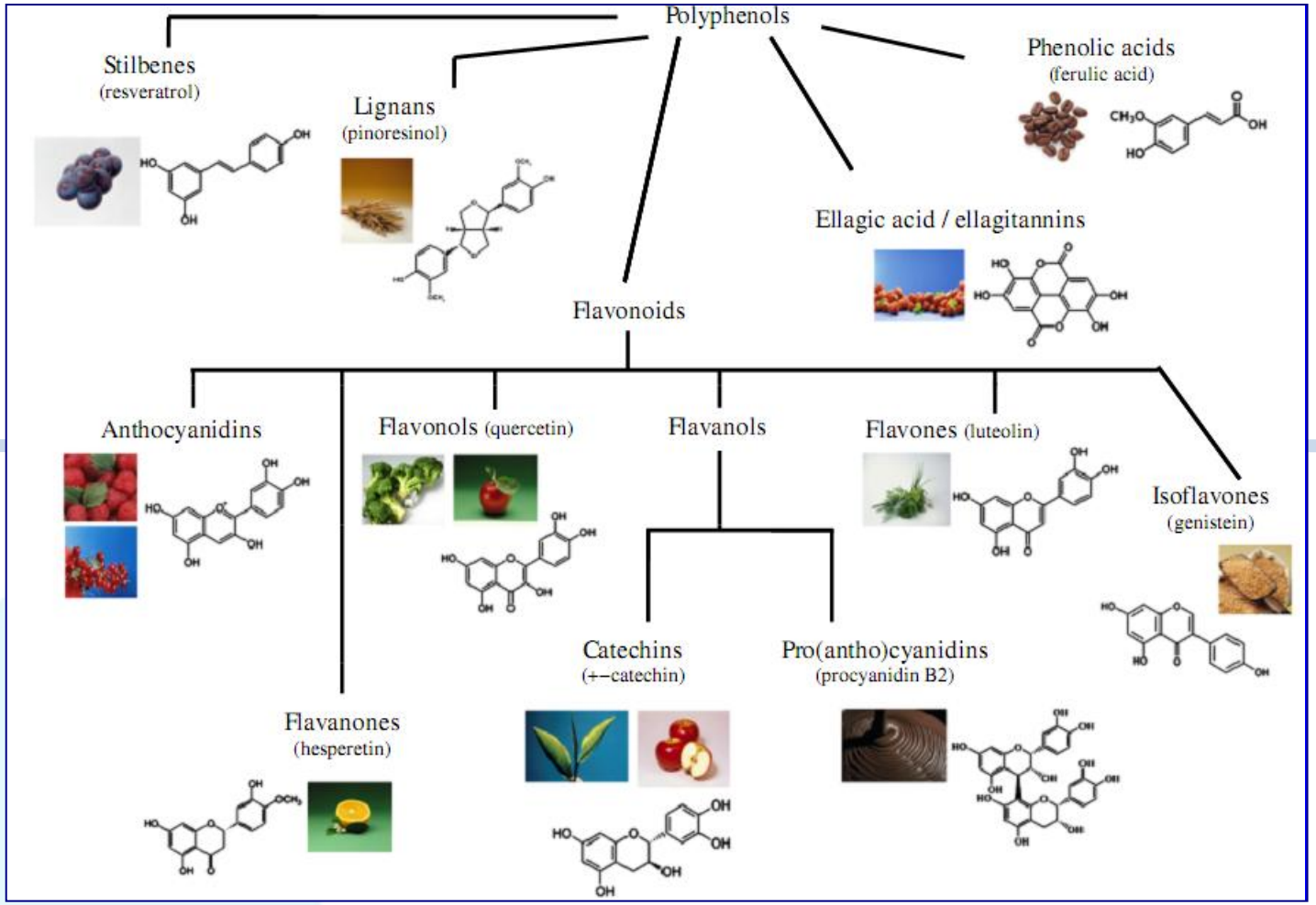












Different classes of polyphenols, their known plant and fruit sources

Source: Clin Exp Allergy, 41, 1346–1359

2006第一个植物药Veregen获FDA 准，德MediGene公司开发,由Bradley制药公司负责美国市场, 剂型为皮肤外用药。

远古时期，藏族先民便足迹并繁衍生息于海拔4000米苦寒之地-青藏高原。 充分利用高原自然植物、植被供其生存

生态环境是适应与习服的重要因素

强UV - B辐射胁迫时, 植物体内产生的次生代谢多糖、生物碱、生物酸、黄酮类物质可以吸收大量UV- B辐射而改变其穿透率,如同形成了一道天然屏障,对 U - VB辐射有直接的减缓作用。,

这些次生代谢物还对其它生物带来深刻的影响, 例如对昆虫起到抑制作用，增强的 UV - B辐射对麦田生态系统中种群数量动态的影响、防止草食动物过度摄食以及影响动物幼体发育和抵御病毒侵染。

植物体内次生代谢产物的改变可能会改变草食动物的摄食偏好。

植物黄酮的生物活性：

Estrogen-like activity

Direct influence on extracellular matrix

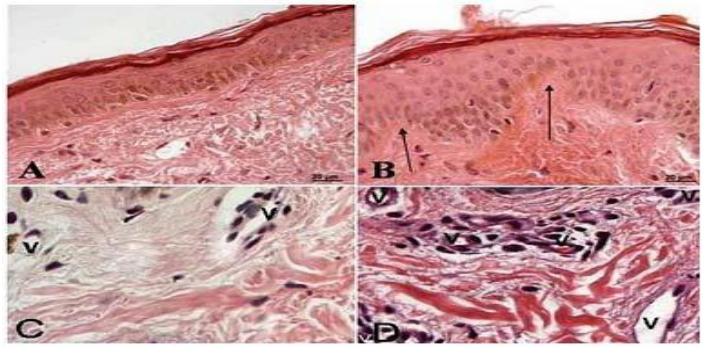
Cardiovascular protective effect

Anti-inflammatory and anti-allergic effect

Photoprotection

Anti-microbial activity

Anti-oxidative stress activity



Clin Dermatol, 2008, 26, 347–357

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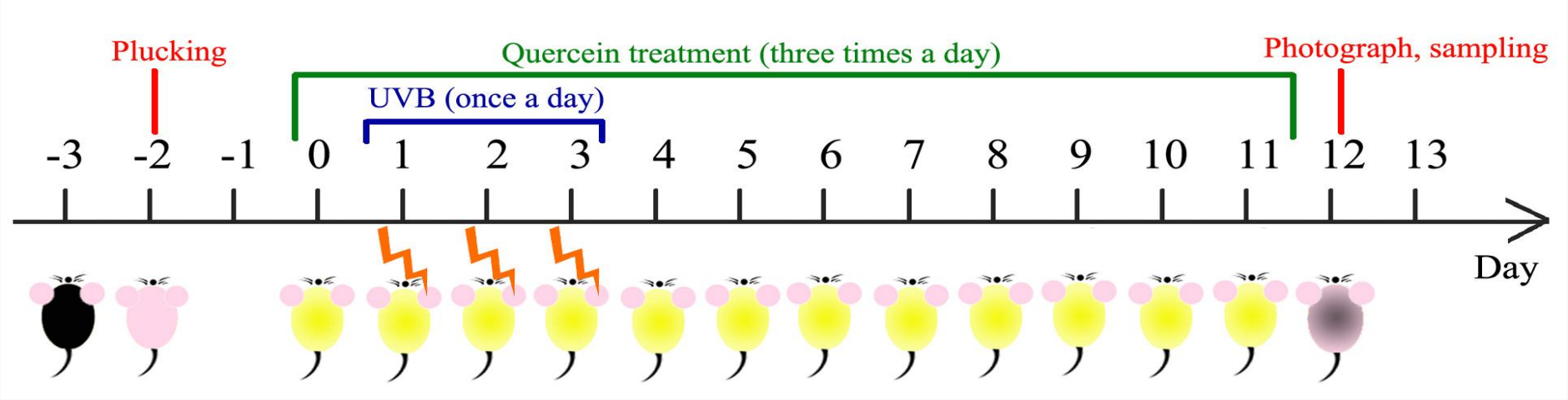
2 我们的研究

动物模型：

- C57/BL，Female，7 wk
- 4组：control, Qu, UVB, UVB+Qu
- QU膏剂浓度约0.5 mg/g
- UVB强度约为90 uW/cm^2（297 nm下），每次照射150 s



药效评价：

- Photograph
- 观察表皮黑色素分布（Eosin染色）
- 皮肤颜色定量分析（Mean gray value）

  Gray value=0.299 red+0.587 green+0.114 blue

  White: 255

  Black: 0

  每只小鼠背部随机取10个点计算

作用机制研究：

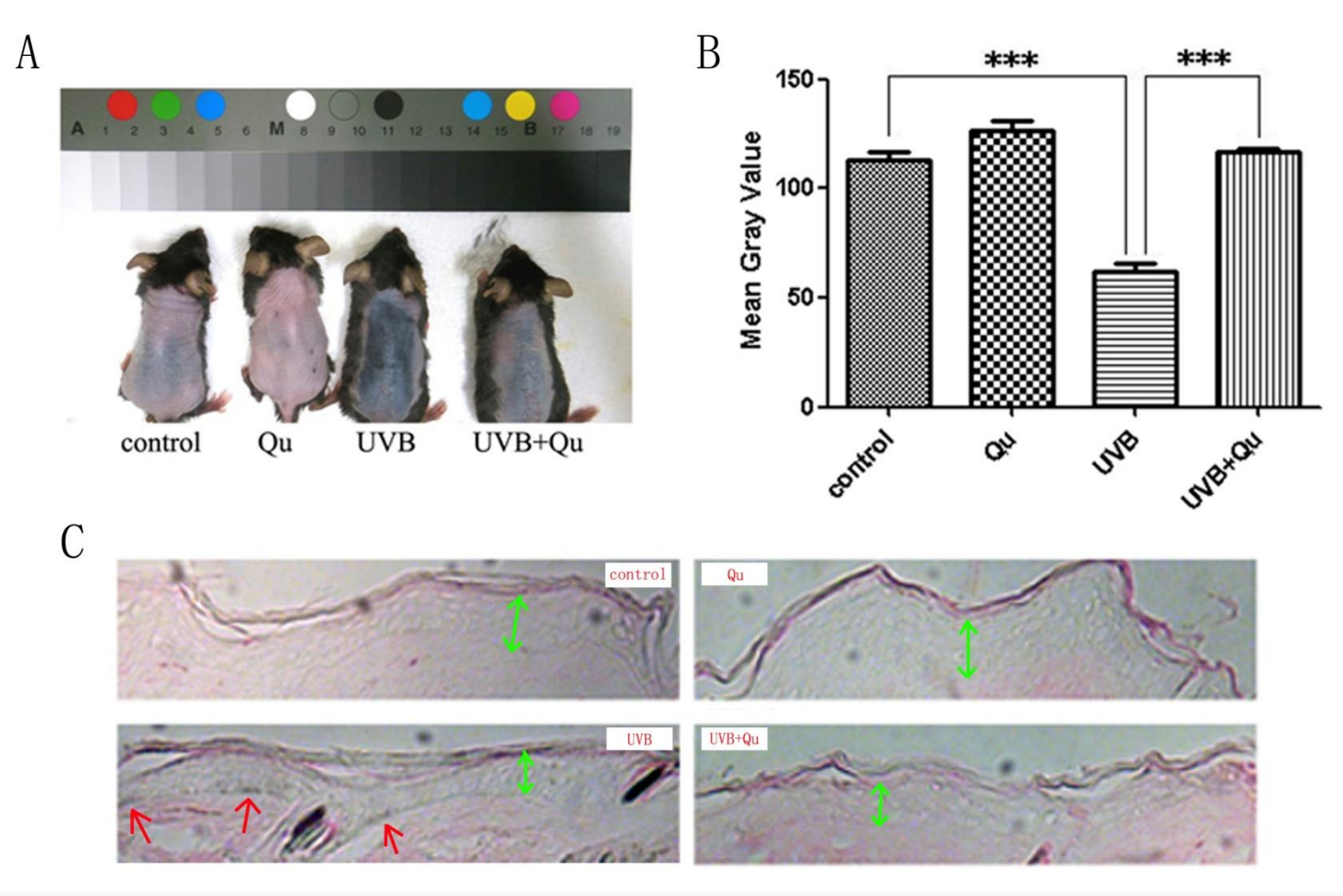
- 体内：表皮alpha-MSH水平（免疫荧光染色、免疫组化）
  表皮黑色素数目（黑色素银染）
- 体外：MITF基因表达（real-time PCR）
  MITF蛋白水平（western blot）
  黑素细胞生长抑制（MTT）
  细胞周期（流式检测）

Melan-a细胞：永生化的小鼠非肿瘤黑色素细胞

2 我们的研究

QU药效：

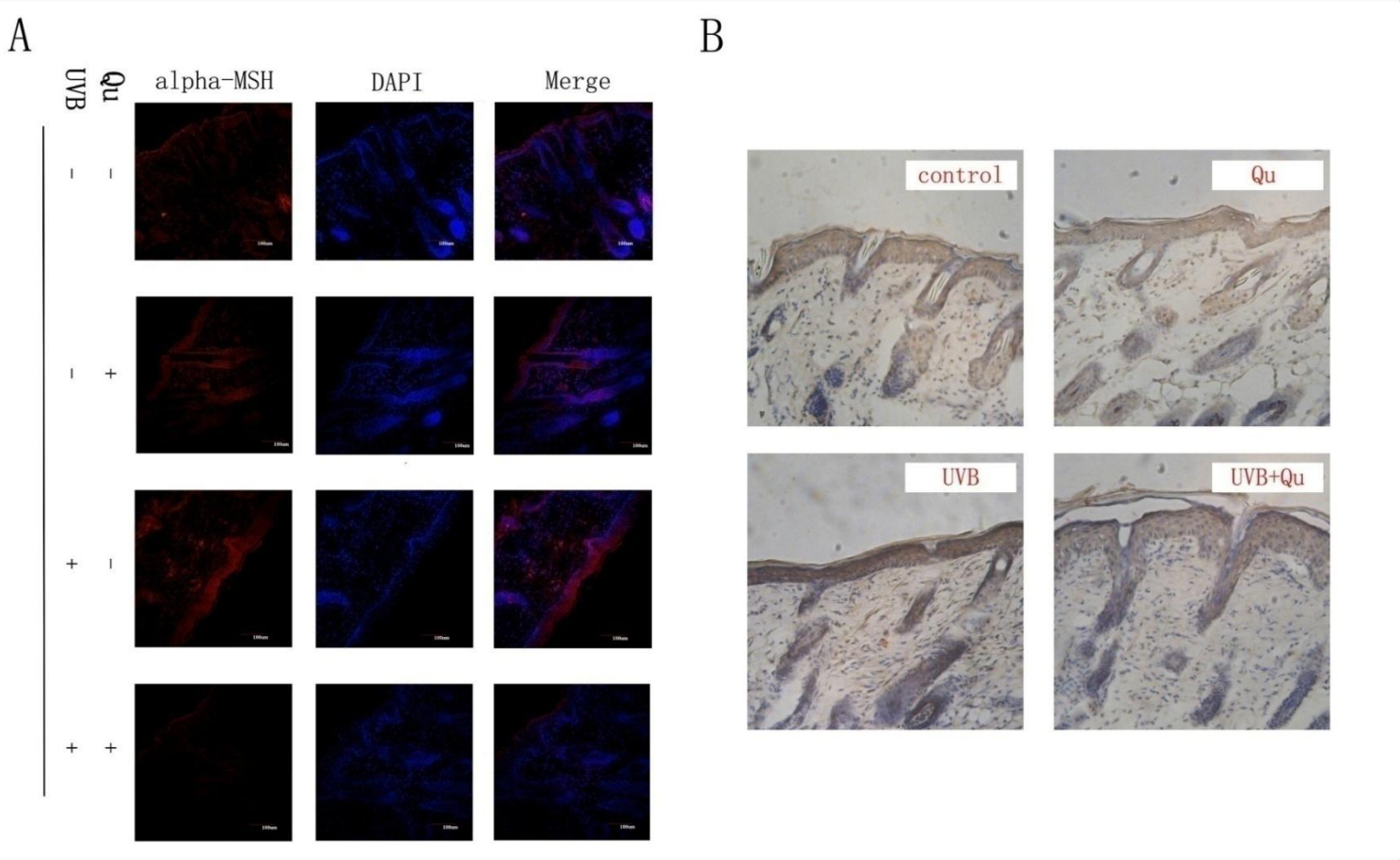
- Photograph
- Eosin染色
- Mean gray value



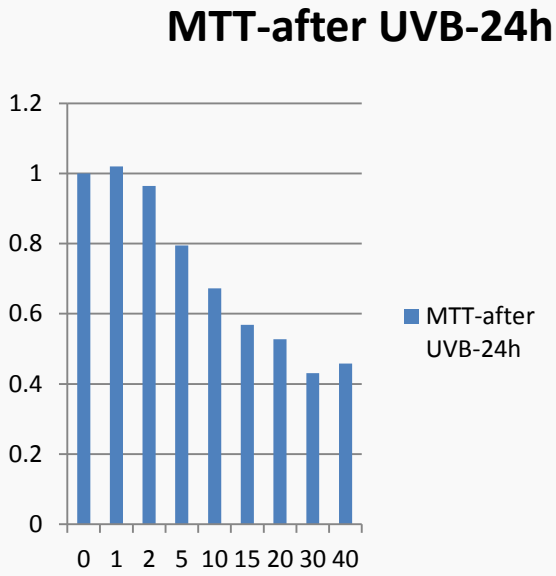
2 我们的研究

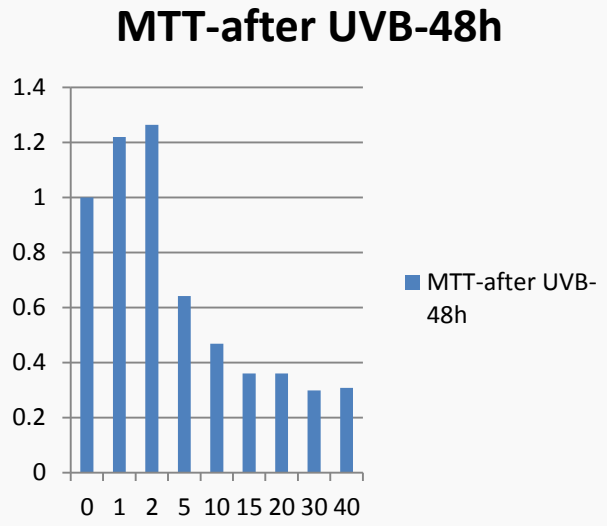
表皮alpha-MSH：

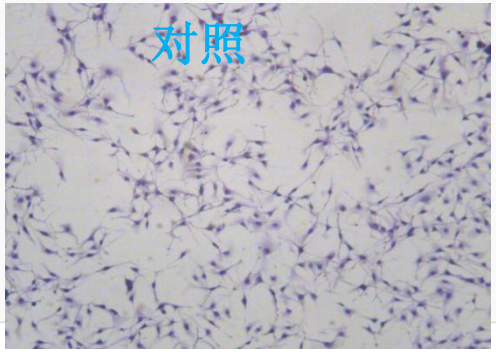
- 免疫荧光染色
- 免疫组化



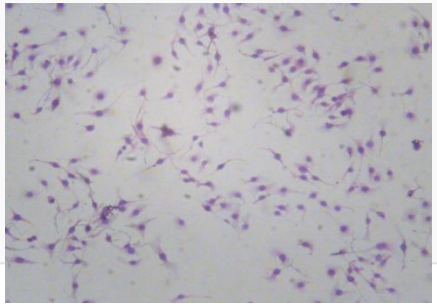
黑素细胞对紫外线应答







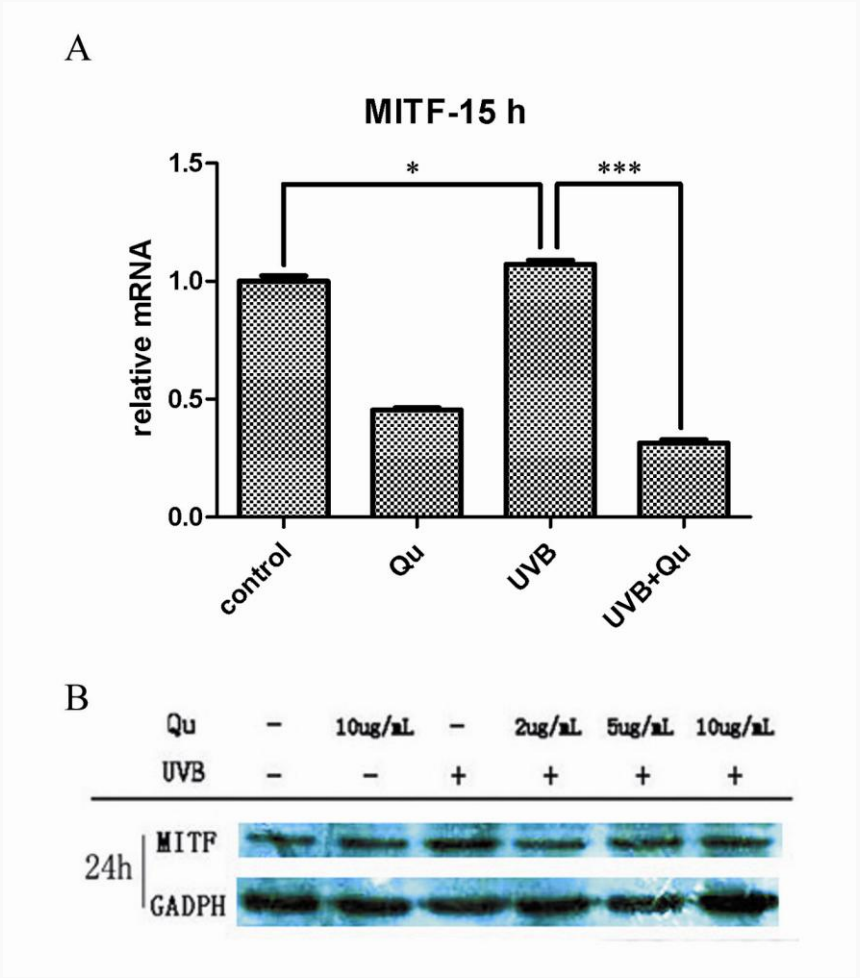
UVB



2 我们的研究

黑素细胞MITF：

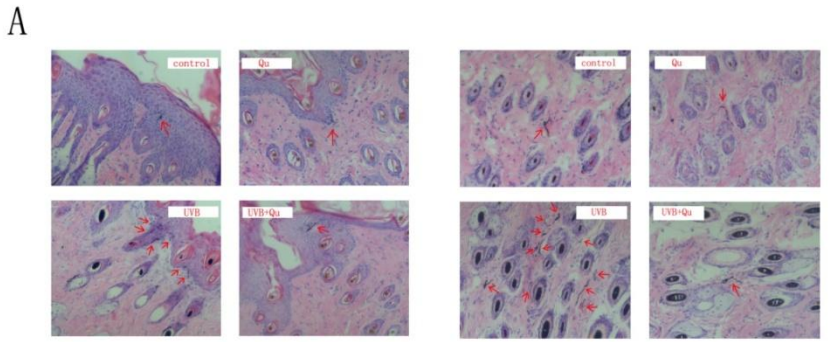
- real-time PCR
- western blot

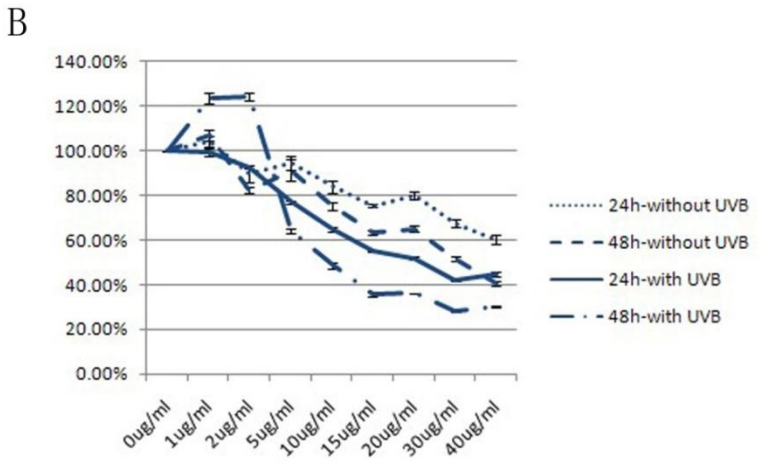


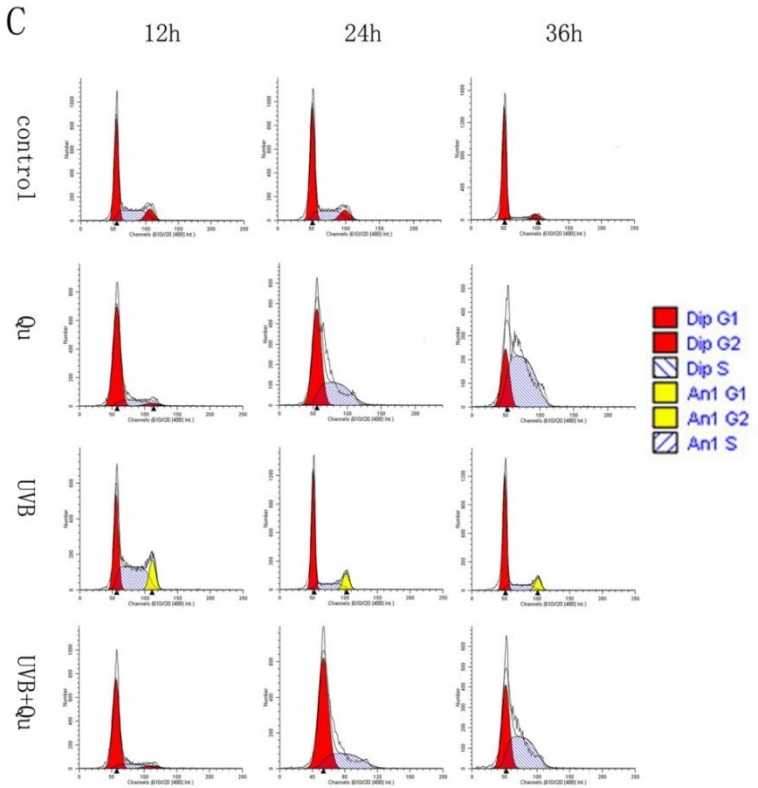
2 我们的研究

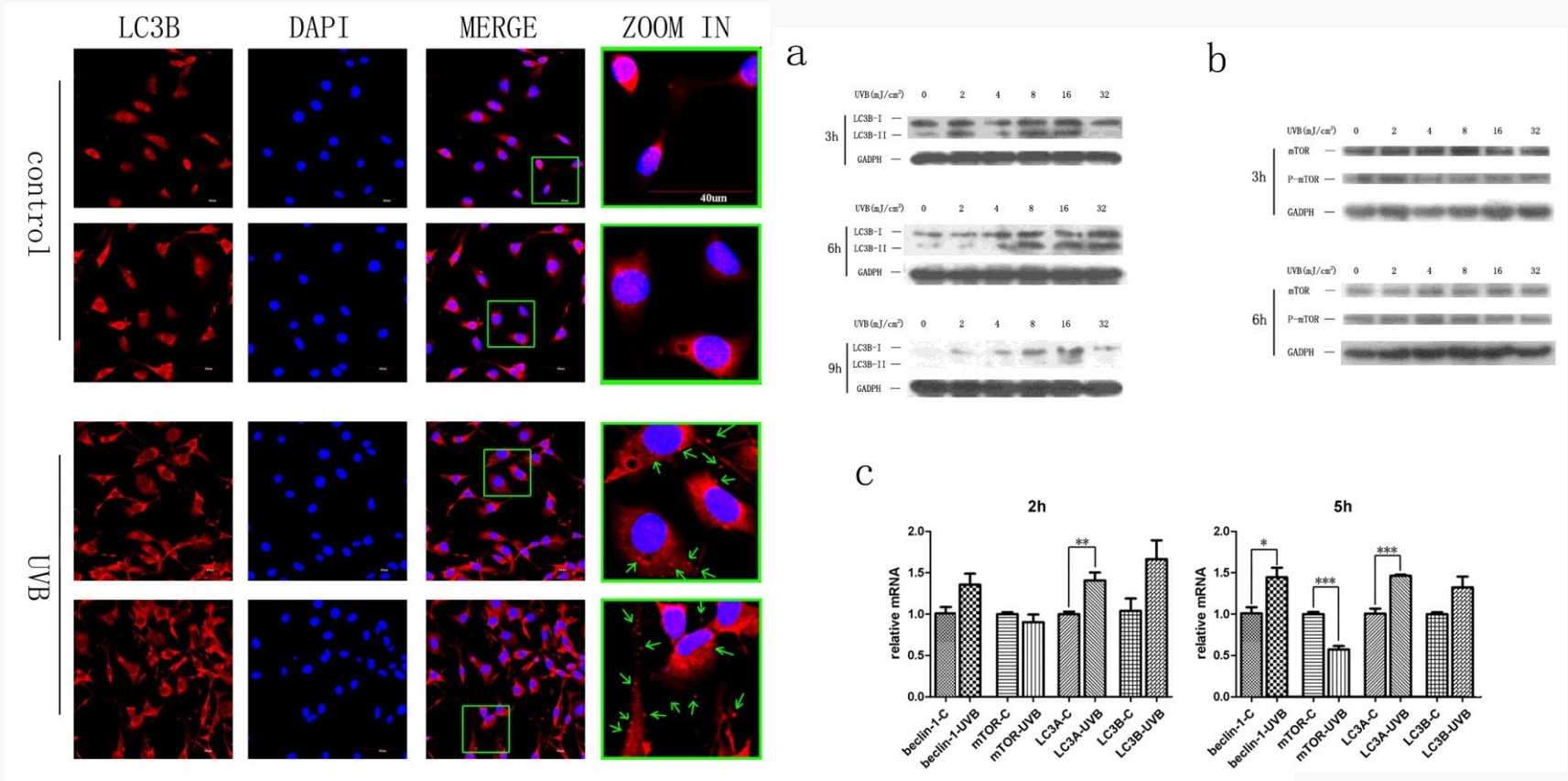
UVB对黑素细胞影响：

- 黑色素银染
- MTT
- 细胞周期







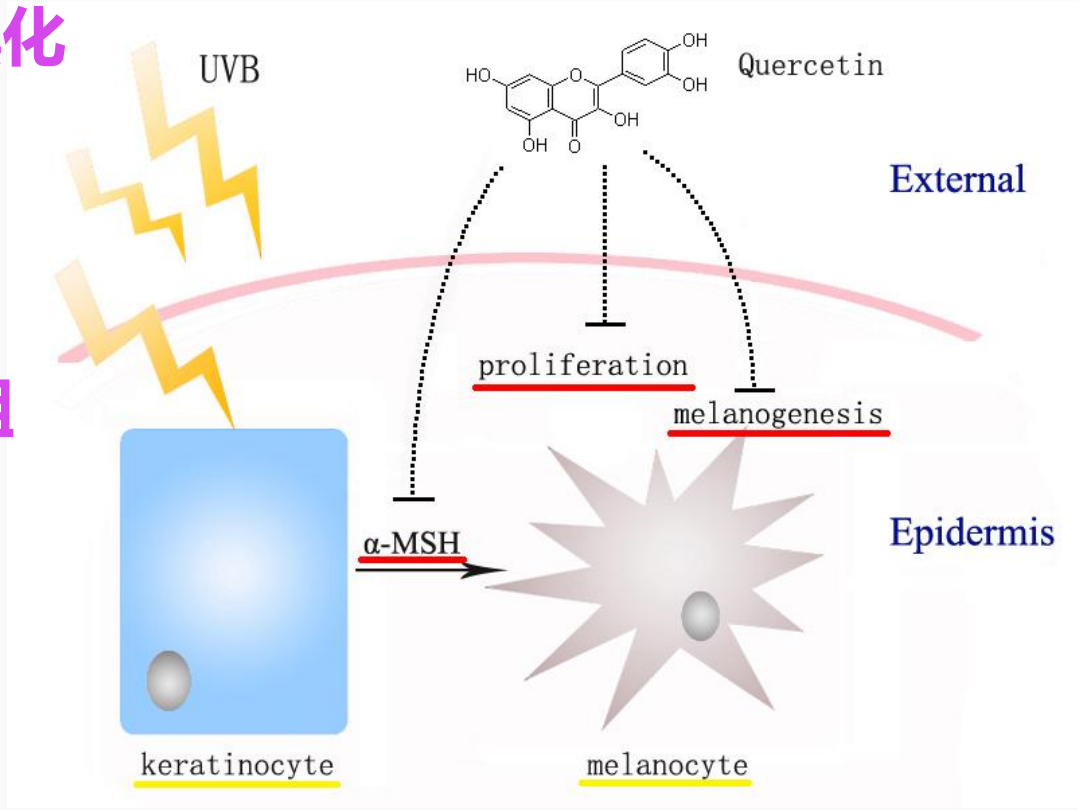


2 我们的研究

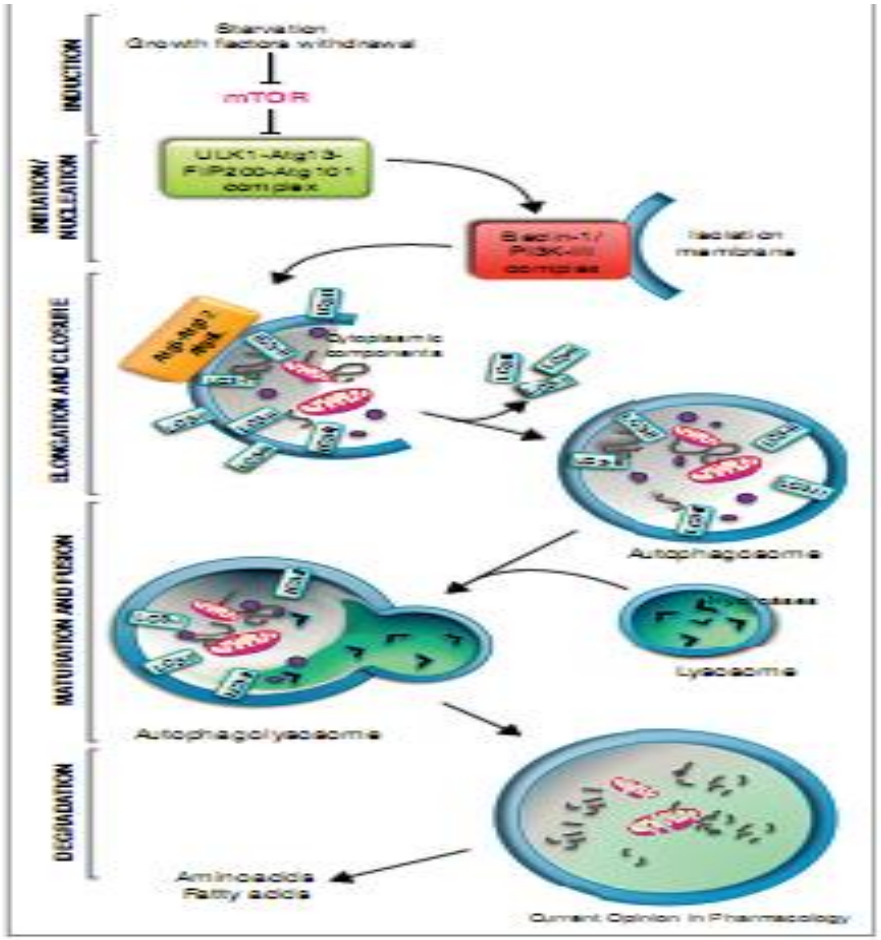
小结：

- QU能够缓解UVB引起的皮肤过黑化
- 作用机制：
  1. QU抑制表皮alpha-MSH
  2. QU抑制黑素细胞MITF
  3. QU抑制黑素细胞有丝分裂，阻断在分裂中期

抑制黑素细胞过度增殖

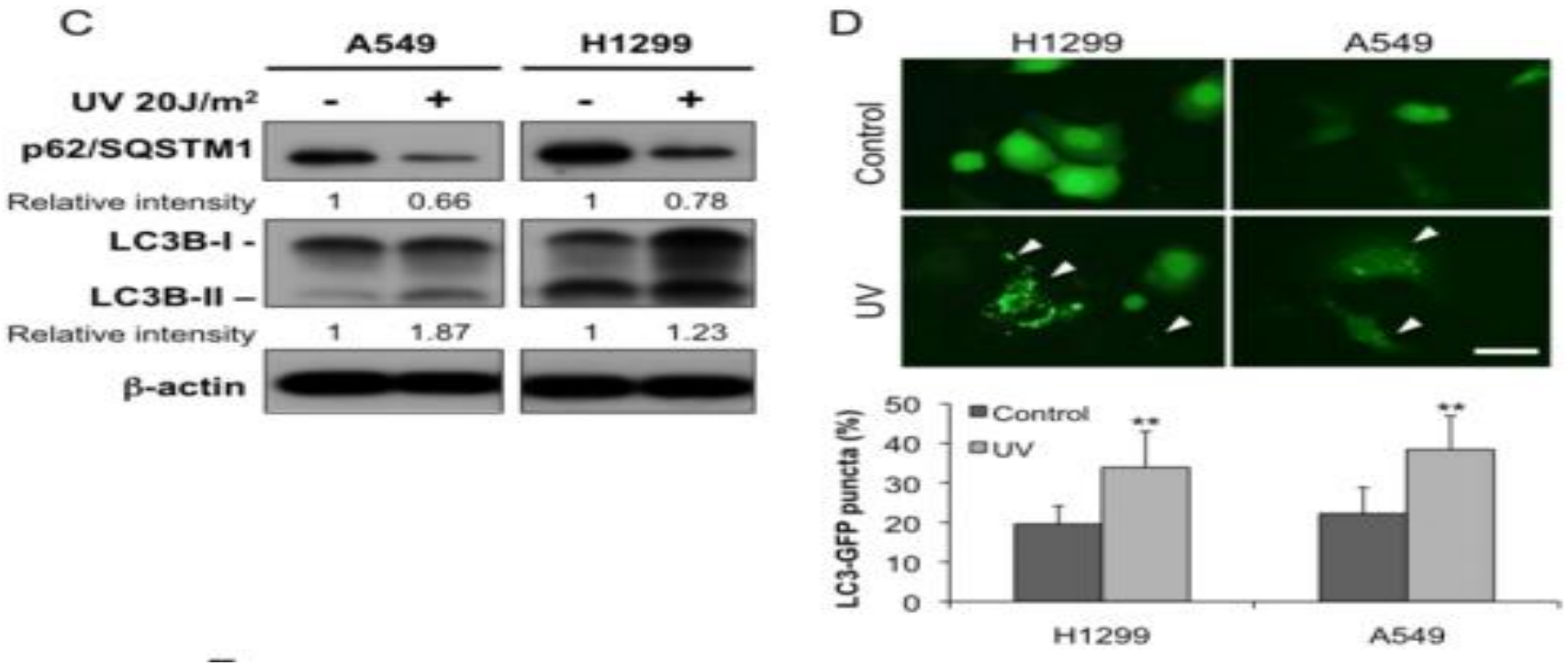


探讨与展望



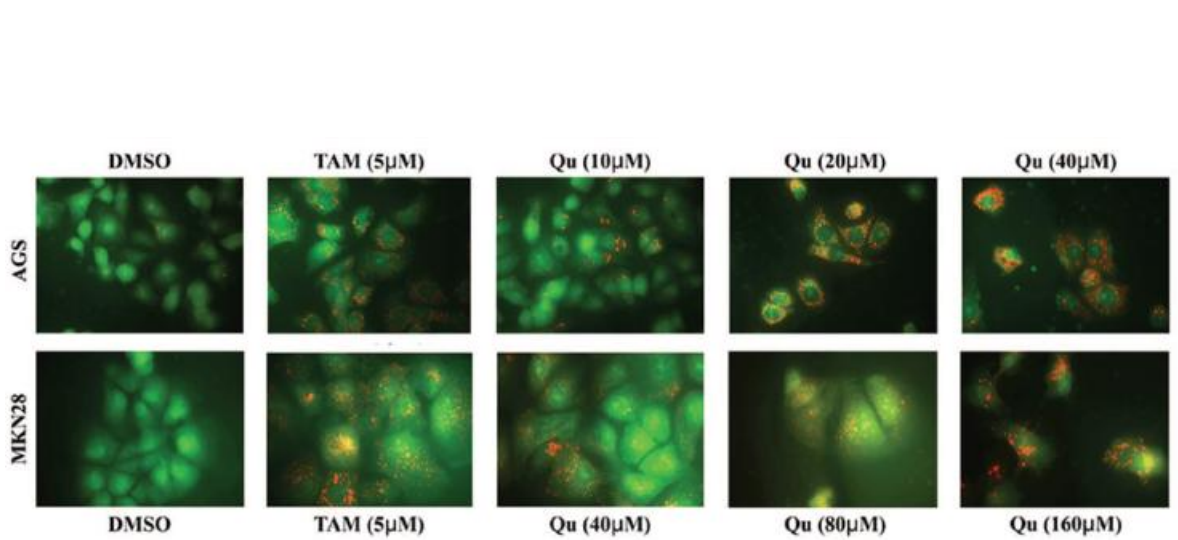
Schematic illustration of the main steps in the autophagic process.

几乎所有的衰老组织都存在溶酶体系统形态学和酶学的改变。随着年龄的增长，生活环境的恶化，细胞内自噬作用开始减弱，导致细胞适应外界环境和自身防御反应的能力降低。细胞自噬恰能帮助细胞清除具有细胞毒性、长时间存活和具有聚集倾向的蛋白质，所以细胞自噬成为降解这些变性蛋白的有效机制(Metcalf DJ,2012)



Targeting Protective Autophagy Exacerbates UV-Triggered Apoptotic Cell Death

Int. J. Mol. Sci. 2012, 13, 1209-1224; doi:10.3390/ijms13011209



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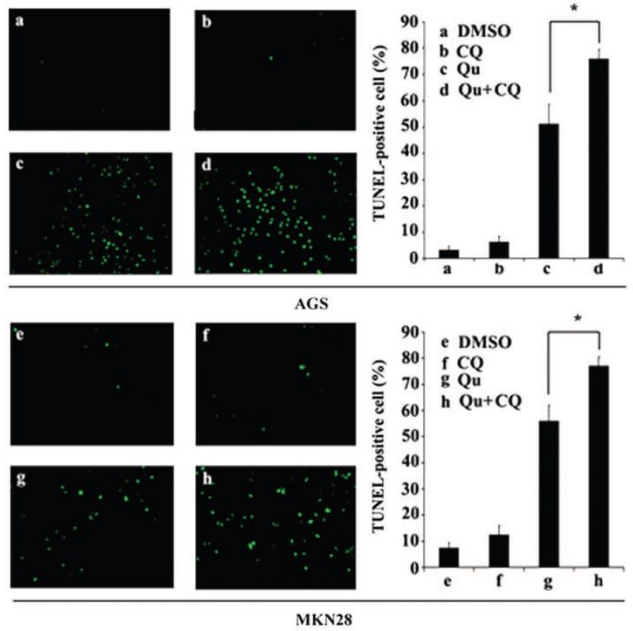
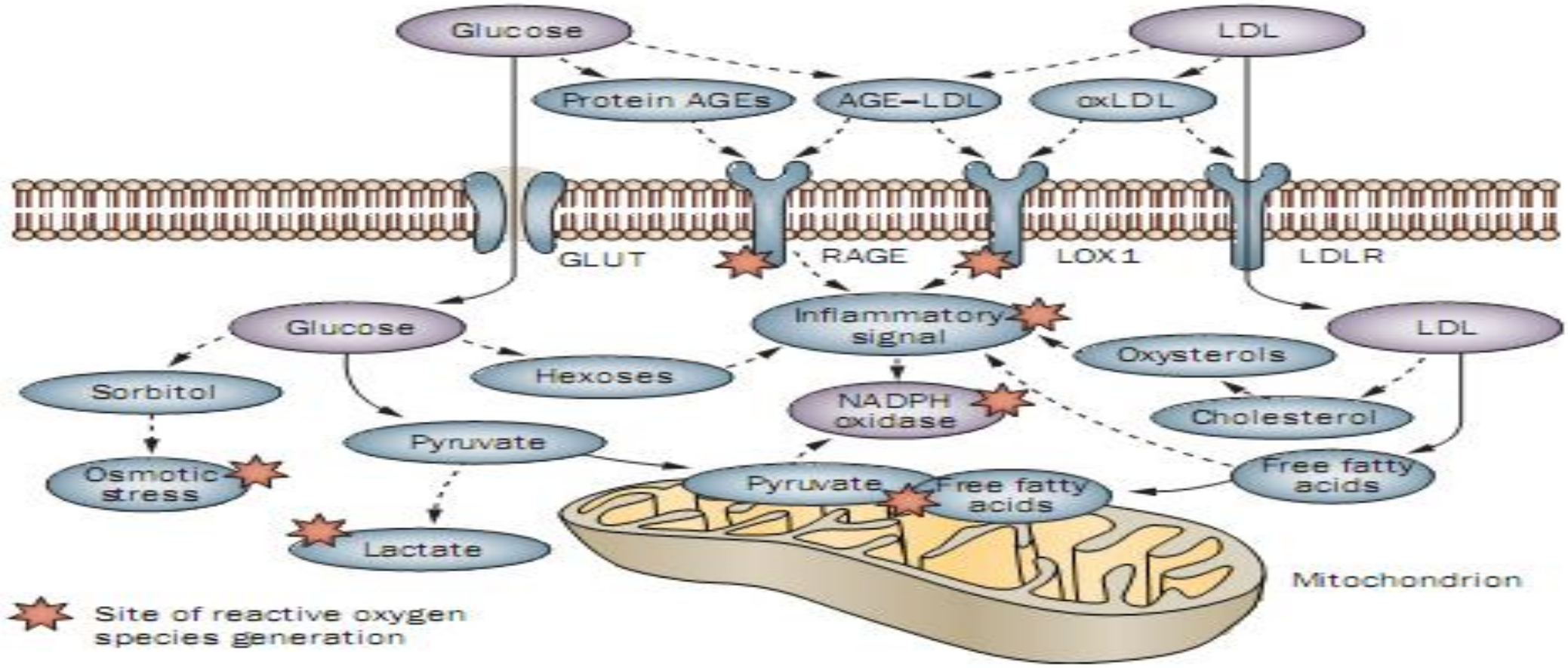


Bioscience Quercetin induces protective autophagy in gastric cancer cells
Autophagy 7:9, 966-978; September 2011;



几乎所有的衰老组织都存在溶酶体系统形态学和酶学的改变。随着年龄的增长，生活环境的恶化，细胞内自噬作用开始减弱, 导致细胞适应外界环境和自身防御反应的能力降低。细胞自噬恰能帮助细胞清除具有细胞毒性 、长时间存活和具有聚集倾向的蛋白质，所以细胞自噬成为降解这些变性蛋白的有效机制(Metcalf DJ,2012)

Anti-inflammatory effect - Quercetin

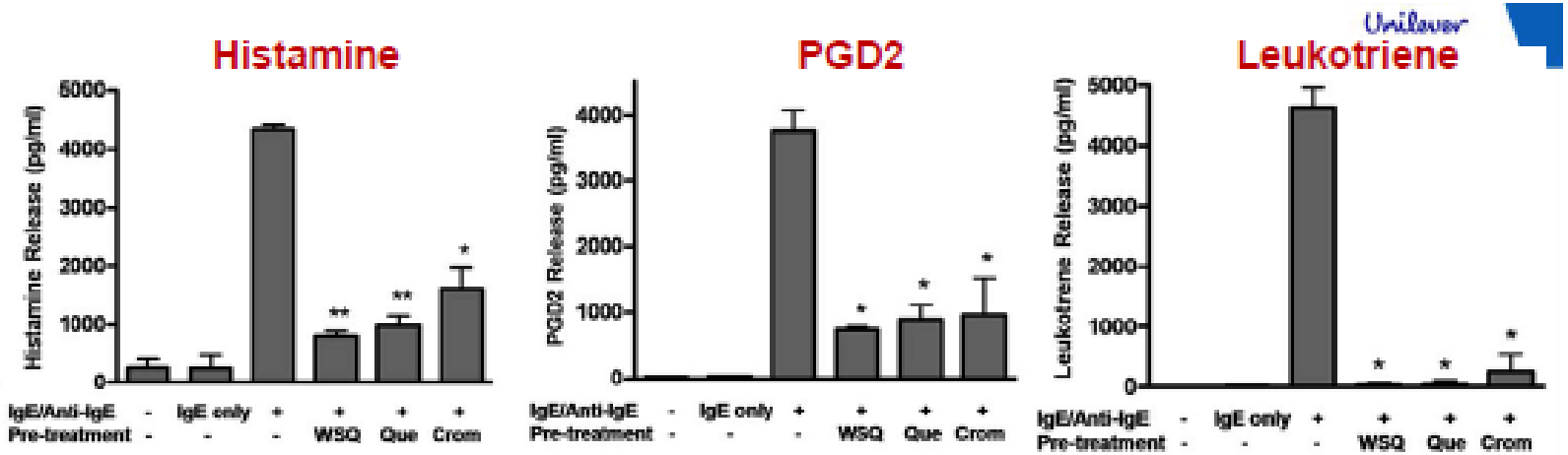


Fig. Quercetin inhibited degranulation of primary human cord blood-derived cultured mast cells (hCBMCs) triggered by IgE/Anti-IgE. * $p<0.05$, ** $p<0.01$. WSQ=water soluble quercetin; Que = quercetin; Crom= cromolyn.

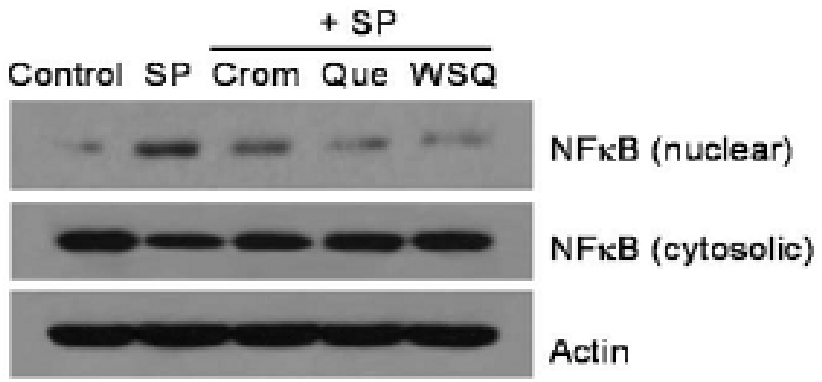
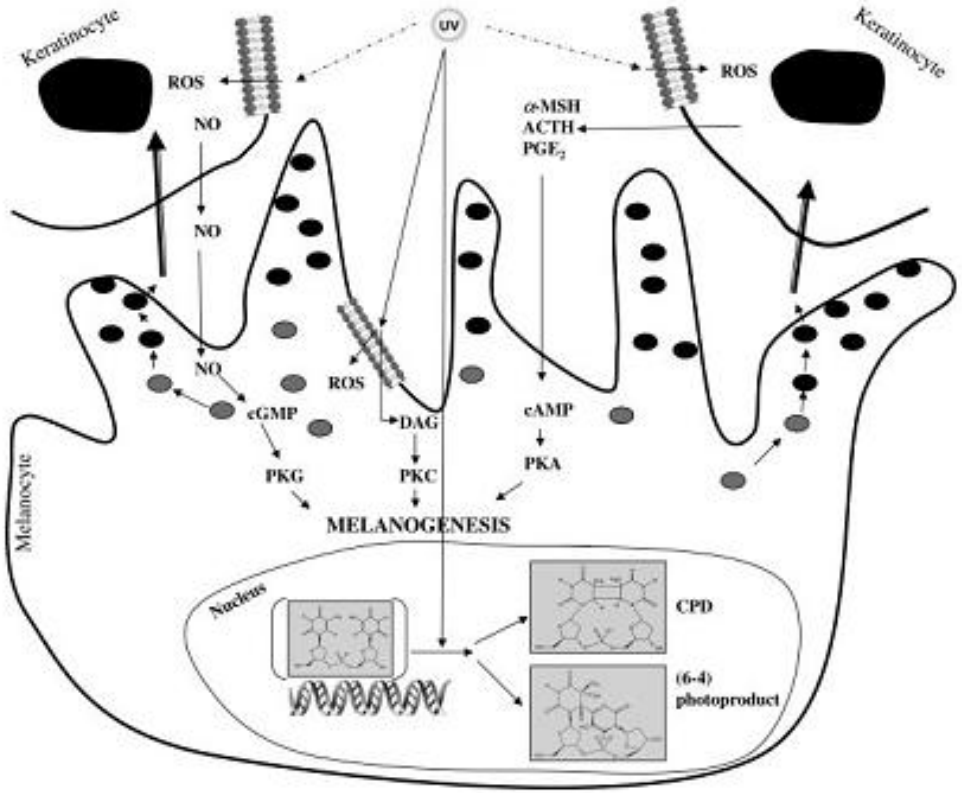
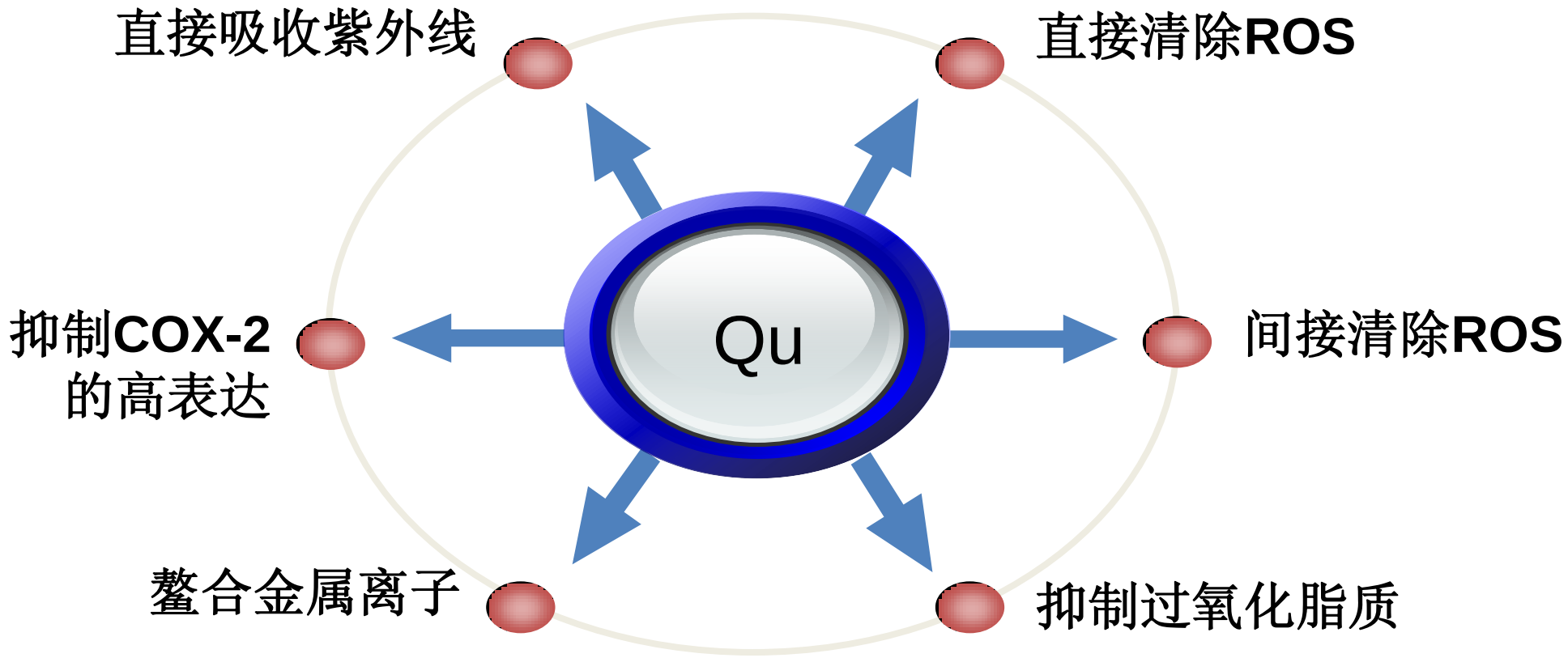


Fig. Quercetin inhibits nuclear translocation of NF-kB induced by Substance P (SP) in LAD2 human mast cells.

Source: PlosOne, 2012, 7: e33805



. Mechanisms involved in the (hyper)-pigmentation induced by UV-R. The tanningresponse is determined by a complex set ofregulatory processes involving direct effects ofUV-R on melanocytes and indirect effects through the release of keratinocyte-derived factors.


直接吸收紫外线
直接清除ROS
抑制COX-2
的高表达
Qu
间接清除ROS
螯合金属离子
抑制过氧化脂质

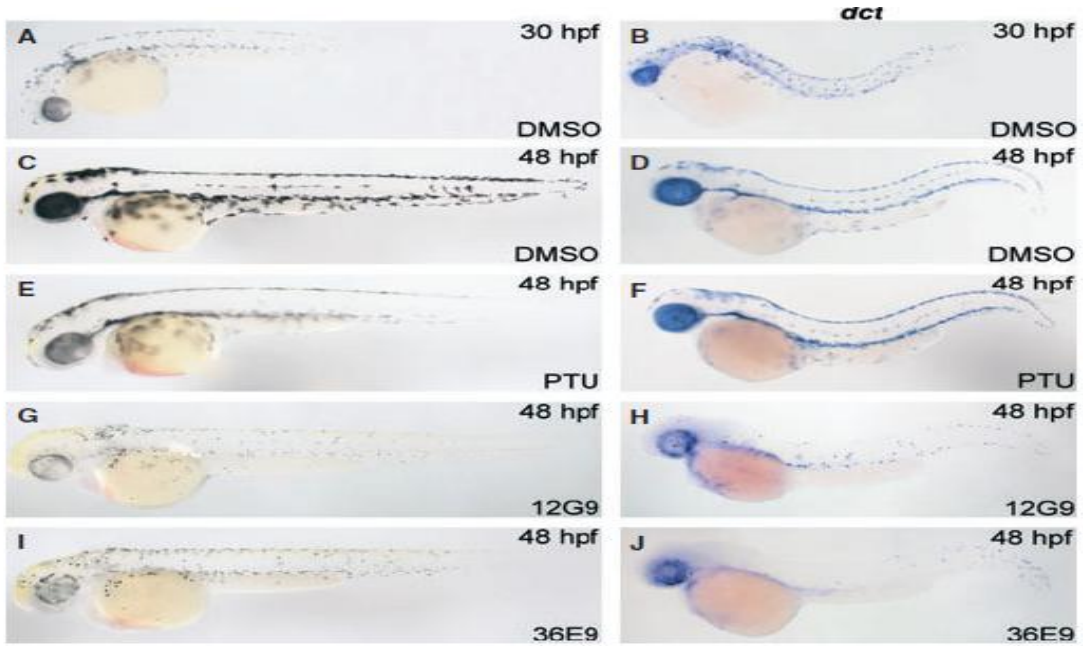


Figure 3. 12G9 and 36E9 induced morphological changes in differentiated melanocytes. All embryos were treated from 30 to 48 hpf except in A and B. Lateral view, anterior to the left and dorsa to the top. A, C, E, G, and I show the pictures of live embryos; B, D, F, H, and show the *dct* in situ hybridization. (A and B) 30 hpf embryos before treatment. (C and D) Embryos treated with DMSO had stellate melanocytes, and the pattern of *dct* positive cells matched the melanocytes. (E and F) Embryos treated with PTU had less melanin, and the *dct* expression pattern was normal. Embryos treated with compound 12G9 (G and H) o 36E9 (I and J) had less pigmentation, condensed melanocytes, and severely reduced number of *dct* positive cells.

剂型修饰

促进药物透过皮肤角质层的机理
1.水合作用
2.穿透机制
3.融合机制
4 .局部作用

皮肤黏膜药物传递系统的研究与评价

功能性单体化合物纳米
脂质体经皮给药系统的研究

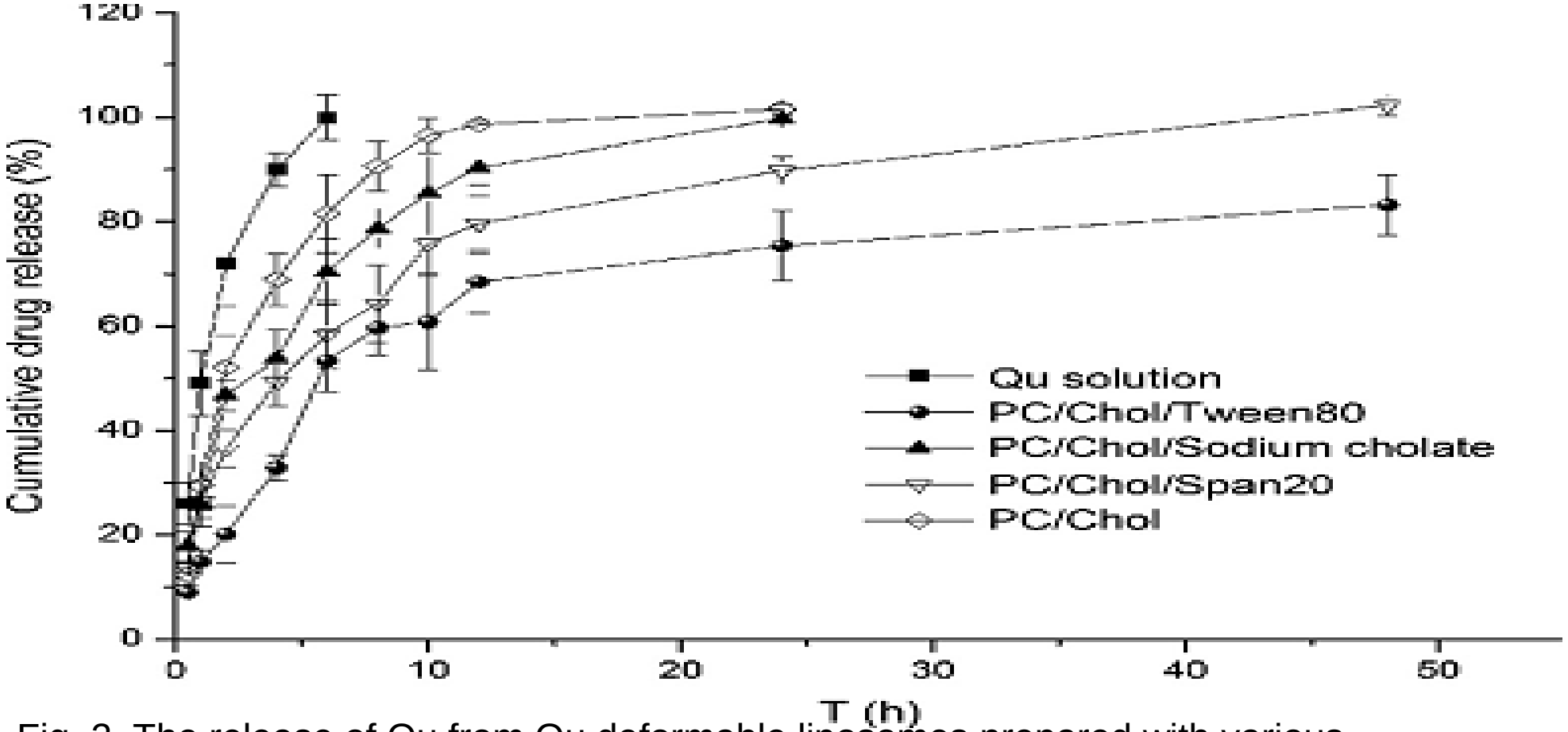


Fig. 2. The release of Qu from Qu deformable liposomes prepared with various surfactants

D. Liu et al. / Journal of Photochemistry and Photobiology B: Biology 127 (2013) 8–17

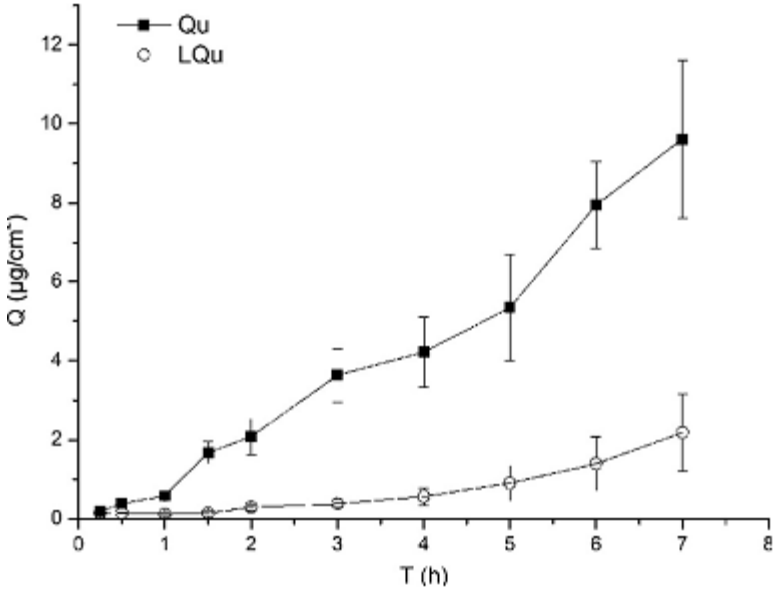
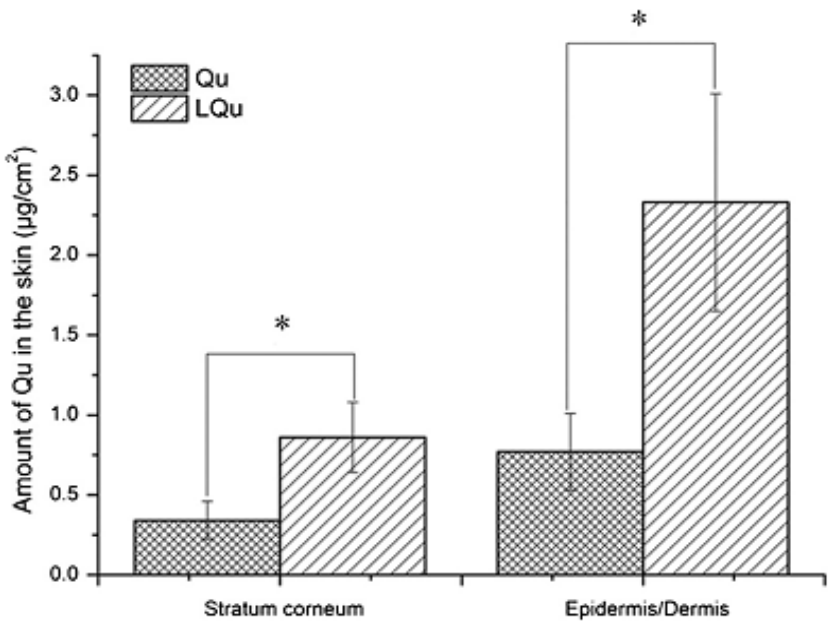
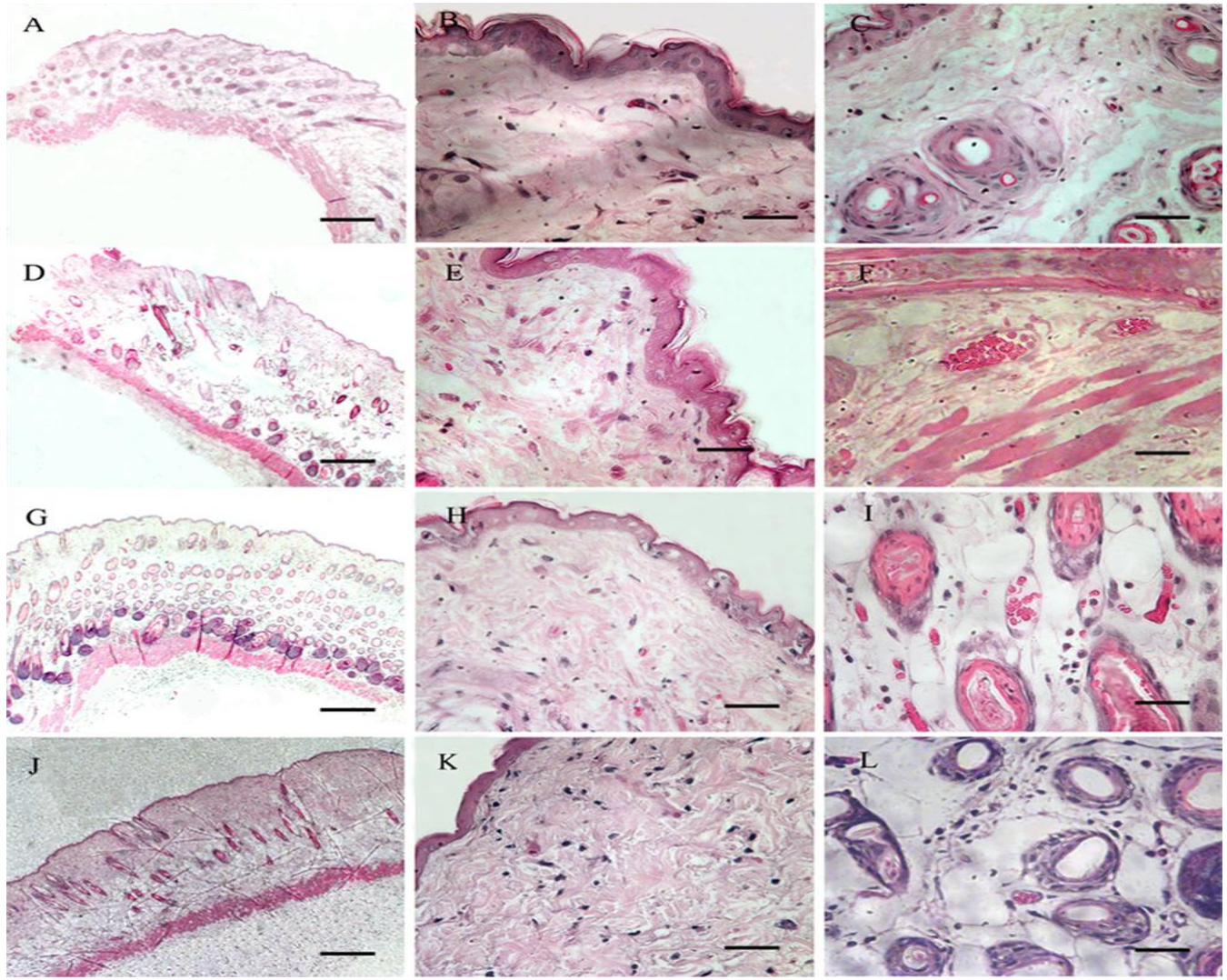


Fig. 7. The in vitro skin permeation profiles of Qu deformable liposomes (LQu) and Qu suspension (Qu), (n = 3).



Amount of Qu retained in skin in an in vivo skin deposition study after applying Qu deformable liposomes or control Qu solution. Each value is the mean ± S.D. (n = 3),) indicating $P < 0.05$.

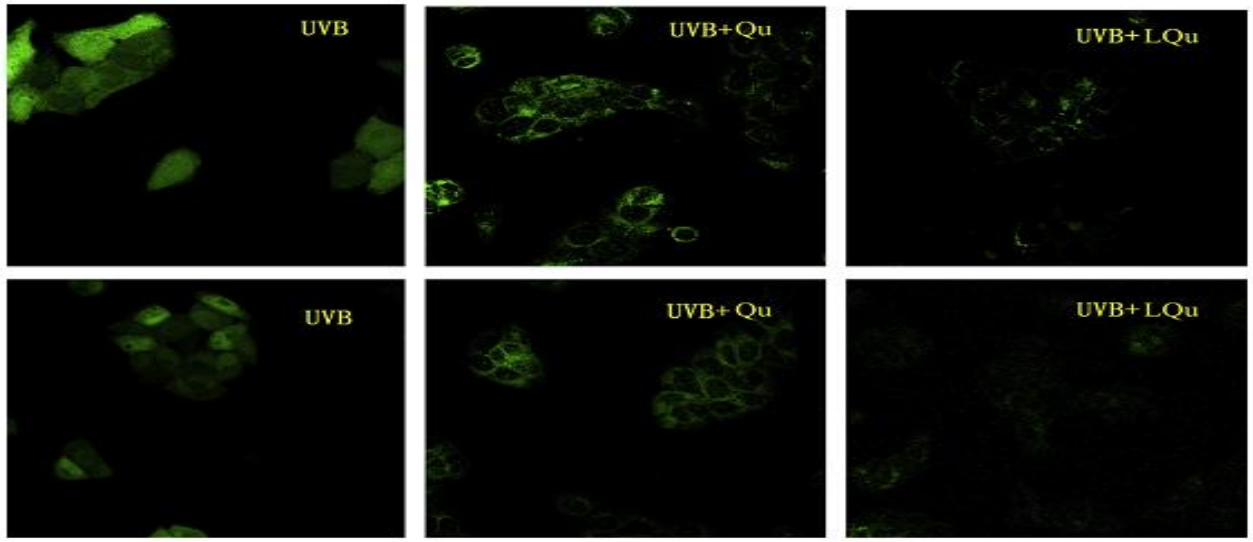


正常组(A,B,C)：皮肤无水肿现象，角质层致密，结构完整，颗粒层清楚，胶原纤维排列有序，无明显炎性细胞，毛细血管无充血。

UVB模型组(D,E,F)：水肿明显，角质层不完整，颗粒层细胞发生损伤，胶原纤维断裂明显，排列混乱，炎性细胞增多。

Qu溶液组(G,H,I)：皮肤有水肿现象，角质层较完整，颗粒层细胞有损伤，胶原纤维有断裂，排列欠整齐，可见炎性细胞，毛细血管有充血。

Qu脂质体组(J,K,L)：皮肤有轻微水肿，角质层结构完整，颗粒层细胞无损伤，胶原纤维排列有序，可见少量炎性细胞，毛细血管充血不明显。


UVB
UVB+Qu
UVB+LQu
UVB
UVB+Qu
UVB+LQu


ISASI

清華大學
Tsinghua University

Thanks