Detection and quantitation of 5-s-cysteinyldopa in melanotic and amelanotic melanoma in comparison with nonpigment cell tumors and it's urinary excretion ; なし ; Experimental and…

Manoj, Vinoy, Mojamdar

(Degree)
博士（医学）

(Date of Degree)
1983-03-31

(Resource Type)
doctoral thesis

(Report Number)
甲0429

(URL)
https://hdl.handle.net/20.500.14094/D1000429

氏名・（本籍）	Manoj (マノジ) Vinoy (ビナイ) Mojamdar (マジャンダー)　（インド）
学位の種類	医学博士
学位記番号	医博い第391号
学位授与の要件	学位規則第5条第1項該当
学位授与の日付	昭和58年3月31日
学位論文題目	Ⅰ DETECTION AND QUANTITATION OF 5－S－CYSTEINYLDOPA IN MELANOTIC AND AMELANOTIC MELANOMA IN COMPARISON WITH NONPIGMENT CELL TUMORS AND IT'S URINARY EXCRETION （悪性黒色腫（melanotic と amelanotic 型）ならびに非色素細胞腫瘍内および同患者尿中の5－S－cysteinyldopa の同定，定量） Ⅱ 悪性黒色腫の各 stage における尿中5－S－cysteinyldopa 値ならびに dopa 負荷法の試み Ⅲ Experimental and Clinical Investigation on the Dynamics of 5—S—Cysteinyldopa in Malignant Melanoma *in vivo*：Production, Excretion, and Regulatory factors （悪性黒色腫内の5－S－cysteinyldopa 動態の実験的ならびに臨床的研究：生成，排泄，制御因子） Ⅳ TYROSINASE AND γ－GLUTAMYL TRANSPEPTIDASE IN 5—S—CYSTEINYLDOPA GENESIS WITHIN MELANOTIC AND AMELANOTIC MELANOMAS （Melanotic 型および amelanotic 型悪性黒色腫内5－S－cysteinyldopa 生成に於ける tyrosinase および γ－glutamyl transpeptidase の役割） Ⅴ Effect of DOPA — Loading on Glutathione — dependent 5—S—Cysteinyldopa Genesis in Melanoma Cells *in Vitro* （培養黒色細胞系における glutathione 依存性5—S—cysteinyldopa 生成への dopa 負荷の影響）
審査委員	主査 教授 三島　豊 教授 木幡　陽　教授 溝口史郎

論 文 内 容 の 要 旨

INTRODUCTION

Melanins have been classified into eu- and pheo-melanins. The former are black in color, while the later may be red, brown or yellow. Until recently human and mammalian melanocytes were considered to synthesize only eumelanin, although the existance of pheomelanogenesis was known in birds and other vertebrates. The initial step in both types of melanogenesis was the conversion of tyrosine to dopa and then to dopaquinone by tyrosinase. During eumelanogenesis dopaquinone is converted to dopa-chrome and then to a series of other intermediates which enter into polymerization leading to black eumelanin. In pheomelanogenesis, however, dopaquinone forms either cysteinyldopa directly by the nucleophilic addition of cysteine or indirectly via glutathione-dopa. The cysteinyldopas are then further oxidized to cysteinyldopaquinones, benzothiazine-compounds and then to reddish-brown to yellow colored pheomelanin. With the discovery that cystinyldopas are present in melanomas and is excreted in the urine of both normal and melanoma patients, pheomelanogenesis within human pigment cells has acquired great biological and clinical significance.

IN VIVO STUDIES

I. Detection and quantitation of 5—S—CD in Mongoloid subjects:

Initially the spectrofluorophotometric method of estimation of 5-S-cysteinyldopa(5-S-CD), the major type of cysteinyldopas formed within mammalian pigment cells was standardized. The levels of 5-S-CD in tumors and urine of melanoma patients of Mongoloid origin were estimated and compared with those of non-melanoma subjects. All melanotic melanomas, both primary and metastatic were found to contain substantial amounts of this amino acid. Urinary excretion of 5-S-CD increased with the duration of melanoma. In contrast none of tumors of non-pigment cell origin contained detectable levels of 5-S-CD. Further non-pigment cell tumor bearing subjects showed rather low urinary excretion levels of 5-S-CD. These findings established that 5-S-CD is an excellent biochemical marker for the detection of melanoma and its progression in all human beings irrespective of their race(Publication 1).

II. Melanoma stage and 5—S—CD excretion:

Melanoma patients classified into different stages were investigated for their 24 h urinary 5-S-CD excretion levels. These studies revealed that stage III patients could be easily identified by their high urinary 5-S-CD levels(Mean = 5203.7 μg/24h), it was difficult to identify clearly, on the basis of 5-S-CD excretion values alone, melanoma patients in stage I and stage II from controls.(Publication 2).

III. Dopa-loading test and establishment of an animal model for melanoma:

Based on our in vitro experiments(See below)we devised the dopa-loading test for clearly differentiating stage I and II melanoma patients from control subjects. The values in this test are expressed as percent increase of 5-S-CD excretion after 50mg iv dopa-loading as compared to before dopa-loading.

In control individuals the percent increase of 5-S-CD excretion was found to be only 40.0% in contrast to stage I melanoma patients where the percent increase was 128.7%. However, in stage III melanoma patients the increase in 5-S-CD urinary levels after dopa-loading was only 70.45%. This could be due to the comparatively low concentrations of dopa shared by a large number of metastasized melanoma cells. In view of the clinical limitations on the use of dopa, an Hamster-bearing Greene's melanotic melanoma model has been established. Dopa-loading of 500mg/g body weight injected intraperitoneally led to its complete utilization by the melanoma cells and an increase of 145.8% in 5-S-CD excretion was found(Publication 3).

IN VITRO STUDIES :

IV. Tyrosinase and γ-glutamyl transpeptidase in the synthesis of 5-S-CD:

In addition to clinical aspects of 5-S-CD, enzymes and factors involved in the synthesis of this amino acid has been investigated. B-16 murine, Greene's hamster, and human melanoma cells have been found to secrete substantial quantities of 5-S-CD into their medium. In contrast amelanotic melanoma and non-pigment cells did not secrete this amino acid. In the presence of sodium diethyldithiocarbonate, a tyrosinase inhibitor and iodoacetamide, a γ-glutamyl transpeptidase inhibitor, the melanotic melanoma cells failed to produce and secrete 5-S-CD. These findings indicate that the above two enzymes are involved in the biosynthesis of this amino acid. In further support of this, we have found that amelanotic melanoma, which does not contain 5-S-CD and does not produce 5-S-CD in culture, is poor in both tyrosinase and γ-glutamyl transpeptidase(Publication 4).

V. Effect of dopa and glutathione on 5-S-CD genesis:

Since cysteinyldopas are conjugates of dopa and cysteine, the effects of dopa, cysteine, and glutathione on 5-S-CD genesis by melanoma cells cultured in cystine-free media away from the intricate influences of the whole biological being were investigated. Individually none of these chemicals enhanced the secretion of 5-S-CD into the medium of cultured melanoma cells. In the presence of dopa and cystine media incubated with and without melanoma cells showed the presence of large amounts of 5-S-CD. In the presence of dopa and glutathione, however, only the media containing melanoma cells were found to contain 5-S-CD. Further in contrast to in vitro non-cellular tyrosinase systems where the optimum condition for 5-S-CD production was 1:2 for dopa:thiols on the molar basis, in our cell culture system it was 1:1. This finding of higher concentrations of dopa requirement for producing optimum conditions for 5-S-CD yield in our cell culture system indicates that both eu- and pheomelanogenesis are taking place simultaneously within melanoma cells(Publication 5).

Further work as to the premelanosome as the actual site of 5-S-CD genesis and thus pheomelanogenesis has also been carried out. Taken together, our findings indicate that the regulatory mechanisms involved in the production of eu- and pheo-melanin according to genetically pre-determined proportions may largely be due to the availability of free sulfydryl compounds within pigment cells.

論文審査の結果の要旨

甲392号 Manoj Mojamdar の論文審査の結果につき御報告申し上げます。論文審査にあたっては溝口教授，木幡教授の御協力を得ました。

論文のテーマは「悪性黒色腫細胞内に於ける5－S－cysteinyldopa 生成機序とそれによる悪性黒色腫転移の生化学的診断法の研究」であります。

ヒト色素細胞内にも最近になり，従来より知られている黒色でアルカリ難溶性の eumelanin のみならず，動物のみに知られていた黄色でアルカリ易溶性の pheomelanin も生成され，ヒトにおける皮膚および毛髪等の色調の差は此等共存による eumelanin と pheomelanin の混在比の差によることが明らかとなりつつある。本論文に於ては新たに次の事を解明した。

〔Ⅰ〕Melanin生成過程におけるtyrosineよりdopaさらにdopaquinone までの代謝過程は両種 melanin 共に共通で tyrosinase 酵素により行われるが，その後 eumelanogenesis と pheomelanogenesis の両経路へと分かれる。後者の pheomelanin 生成は cysteine と glutathion の関与のもとに主要中間代謝産物として色素細胞内や尿中にも測定され得る5－S－cysteinyldopa を経て行われる。したがってこの pheomelanin 生成活性の上昇は結果的に eumelanin 生成に制御的に働き，皮膚および毛髪の黒色調の減少を来たす。

〔Ⅱ〕Premelanosome 内に5－S－cysteinyldopa および glutathione 依存性 pheomelanin生成に関与するγ－glutamyltransferase活性の両者の高い局在を認め，ヒトおよび多くの哺乳動物の色素細胞内に生成される melanosome がeumelaninとpheomelaninのmixed型melanin生成に重要な役割を行っていることを更に明らかにした。

〔Ⅲ〕斯る pheomelanin の主要中間代謝物の5－S－CDがヒト悪性黒色腫内に於て大量に生成されており，その腎クリアランスは非常に高く，その大部分が黒色腫担癌患者の尿中に排泄されていることを見出した。而も悪性黒色腫の進展・転移と共に此の5－S－cysteinyldopaの尿中排泄量は平行的に増加し，治癒と共に減少し正常化することが最近見出され，本症治療上の優れた生化学的マーカーとなり得ることが明らかとなった。

以上本研究は，従来ほとんど行われなかったヒトおよび哺乳動物の悪性黒色腫細胞内における pheomelanin の主要中間代謝物である5－S－CDの生成過程およびそれによる黒色腫転移の生化学的診断について重要な知見を得たものとして価値ある業績であると認める。

よって，本研究者は，医学博士の学位を得る資格があると認める。