ISSN:2790-1688 DOI: 10.56028/aetr.4.1.411.2023

Comparison of functions between CYP24A1and Vitamin D3 through Microarray and pathway analysis

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Abstract. CYP24A1 is a rate-limiting enzyme in the metabolic process of vitamin D, which hydroxylate 25 (OH) D3,1,25(OH)2D3 into 24,25(OH)2D3 and 1α,24,25(OH) 3D3 respectively. After microarray analysis of gene set GSE12449, we found CYP24A1 participate in three important pathways, Focal adhesion, ECM-receptor interaction and antigen process. Vitamin D3 make a significance in several gene ontology process, such as basement membrane, response to wounding, positive regulation of gene expression, iron ion binding, integrin binding and so on. In comparison, CYP24A1 also make a role in several process, such as regulation of apoptotic process, transmembrane transport,fructose metabolic process and lipid transport,defense response to virus function and so on. These results reveal the functional commons and differences between CYP24A1 and Vitamin D3, which may indicate the next step study directions between these two genes.

Keywords: CYP24A1; Vitamin D3; microarray; pathway.

1. Introduction

CYP24A1 is a rate-limiting enzyme in the metabolism of vitamin D, and hydroxylate 25(OH)D3,1,25(OH)2D3 into 24,25(OH)2D3 and 1α,24,25(OH)3D3,meanwhile lose the organism active. Learning activity plays a crucial regulatory role in the anti-tumor effect of 1,25(OH)2D3. studies by have Hu and Zhang shown that CYP24A1 depletion facilitates the antitumor effect of vitamin D3 on thyroid cancer cells(Hu and Zhang, 2018). CYP24A1 is also involved in the regulation of Wnt signaling through inflammatory factors(Haussler *et al.*, 2016).

Vitamin D is a fat-soluble prohormone that exerts important roles in calcium metabolism and homeostasis(Horvath *et al.*, 2010)**.**

It remains unclear of the comparison between CYP24A1 and vitamin D3; therefore, it is important to investigate the commons and differences between these two markers. The antitumor activity of 1,25-D3 is determined by the role of CYP24A1(Hu and Zhang, 2018). The high expression of CYP24A1 has been demonstrated to promote the cancer progress in colorectal cancer(Bareis *et al.*, 2002). Thus, the aim of the current study was to identify the role of CYP24A1 in bone repair and the role of vitamin D3 in colorectal cancer.

2. Results

The 3D structure of CYP24A1 was showed in figure 1 and figure 2.

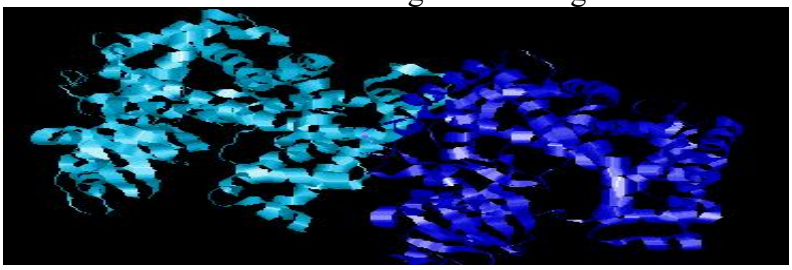

Figure1 The 3D protein structure of CYP24A1 (3K9V.PDB)

ISSN:2790-1688 DOI: 10.56028/aetr.4.1.411.2023

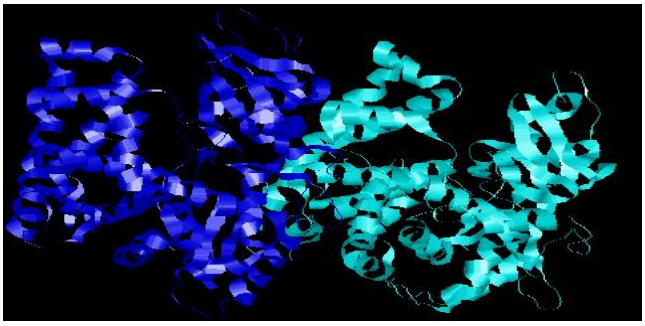

Figure 2 The 3D protein structure of CYP24A1(3K9Y.PDB)

3. Role of vitamin D3 : Microarray analysis of GSE112449 dataset

Scatter plot of gse112449 dataset was showed in figure3.

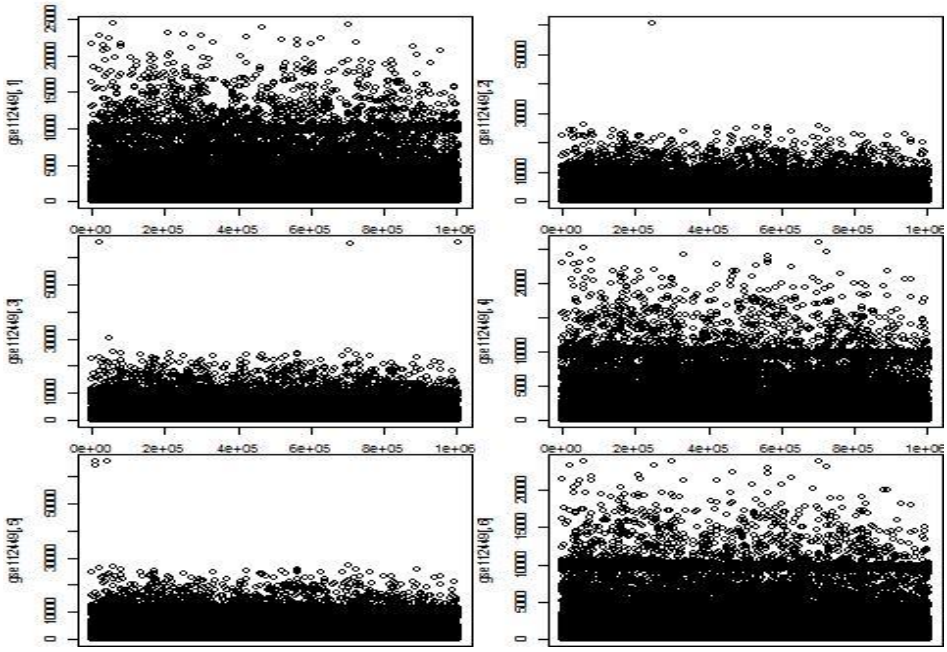

Figure 3 scatter plot of dataset gse112449

Under the criteria of (Fold chang >=2, t-test p value<0.05),136 genes were selected and clustered as 9 branches.(Table 1,Figure 4)

Table 1. filtered genes after cluster analysis categoried as nine branches

Branches	Genes		
Branch1	1415958_at 1417900_a_at 1418987_at 1421735_a_at 1423559_at 1424318_at 1426992_at 1428444_at	1429083_at 1429413_at 1433837_at 1433944_at 1435950_at 1457311_at 1460038_at 1460147_at	1439163_at 1440353_at 1440849_at 1440884_s_at 1448470_at 1448636_at 1448830_at 1451707_s_at 1452474_a_at
Branch2	1415994_at 1416432_at 1416505_at	1424857_a_at 1427213_at 1429918_at 1455739_at	1430738_at 1432001_at 1433965_at 1437030_at 1443855_at

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	1424268_at 1448484_at	1457881_at	
Branch 3	1416431_at 1416818_at 1417244_a_at 1418930_at 1419028_at 1435987_x_at 1439556_at 1440008_at 1452893_s_at 1454395_at	1420357_s_at 1420797_at 1424528_at 1424529_s_at 1424775_at 1425176_at 1431591_s_at 1434777_at 1435091_at	1440021_at 1441995_at 1442467_at 1443018_at 1443273_at 1444706_at 1445787_at 1446653_at 1450813_a_at 1450961_a_at
Branch 4	1417877_at 1418091_at 1419426_s_at 1427556_at 1427591_at 1453657_at 1454720_at 1454838_s_at 1455690_at	1429887_at 1431714_at 1435400_at 1435917_at 1437432_a_at 1438512_at 1438967_x_at 1439669_at 1440084_at 1440435_at	1442025_a_at 1442710_at 1444307_at 1445562_at 1448724_at 1448949_at 1449005_at 1451313_a_at 1451382_at
Branch 5	1418580_at 1421698_a_at 1423555_a_at 1424248_at 1436203_a_at	1447807_s_at 1450047_at 1451912_a_at 1453323_at 1456783_at 1456798_at	1436204_at 1438288_x_at 1439412_at 1440911_at 1442121_at 1460125_at
Branch 6	1418697_at 1419646_a_at	1449398_at 1423328_at	1434449_at 1435605_at
Branch 7	1419391_at	1438540_at	1452520_a_at
Branch 8	1420757_at 1429209_at 1429210_at	1420883_at 1435053_s_at 1446563_at	1420938_at 1449532_at 1450783_at 1456326_at
Branch 9	1423253_at	1433532_a_at	1456228_x_at

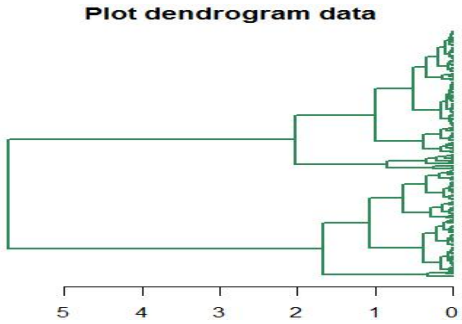

Figure 4 the cluster analysis plot of 146 genes

DAVID analysis results showing Branch 1 genes have positive regulation of apoptotic process, transmembrane transport.Through gene ontology analysis, Branch 2 category found CYP24A1 participate in fructose metabolic process and lipid transport. The BRANCH 3 category genes mainly reveal the defense response to virus function. The Branch 4 also the same analysis and reveals transferase activity and phosphorylation process. Branch 6 genes make a role in response to toxic substance and cytoplasm.

Table 2 Go analysis of branch 1 genes

ISSN:2790-1688 DOI: 10.56028/aetr.4.1.411.2023

Term	P-Value	Benjamini	Branch
positive regulation of apoptotic process	5.2E-2	9.9E-1	1
transmembrane transport	6.0E-2	9.8E-1	1
lipid moiety-binding region:GPI-anchor amidated serine	6.6E-2	1.0E0	1
fructose 2,6-bisphosphate metabolic process	4.6E-3	3.1E-1	2
lipid transport	8.3E-2	9.0E-1	2
defense response to virus	1.1E-2	7.4E-1	3
transferase activity	4.0E-2	9.2E-1	4
phosphorylation	7.0E-2	9.7E-1	4
domain:PH 1	1.7E-2	3.7E-1	5
response to toxic substance	2.4E-2	5.4E-1	6
cytoplasm	4.7E-2	7.5E-1	6

Through gene ontology analysis, Branch 2 category found CYP24A1 participate in **fructose metabolic process and lipid transport.** Pathway analysis showed that CYP24A1 correlate to two important pathways: focal adhesion(hsa04510) and ECM-receptor(hsa04512) interaction(table 3,Figure 5,Figure 6).

Table 3 Pathway analysis resutls of has 04510 focal adhesion: the correlated genes

Term	Genes					
hsa04510 Focal adhesion	ITGB1 ITGA6 FLNA CDC42 MYLK MYL2 DIAPH1 PPP1R12A ROCK1	AKT1 PDPK1 PARVB ILK VCL VASP PTEN PIK3CA TLN1 PIP5K1C	ACTN4 PTK2 CAPN2 PXN ARHGAP5 PRKCA SRC FYN RHOA RASGRF1	CHAD CAV1 BIRC2 BAD BCL2 ELK1 MAPK1 CCND1 MAP2K1 RAF1	HRAS SOS1 GRB2 EGFR EGF SHC1 BCAR1 CRK RAPGEF1 RAP1A	DOCK1 MAPK8 JUN PAK1 RAC1 VAV1 CTNNB1 GSK3B ACTB

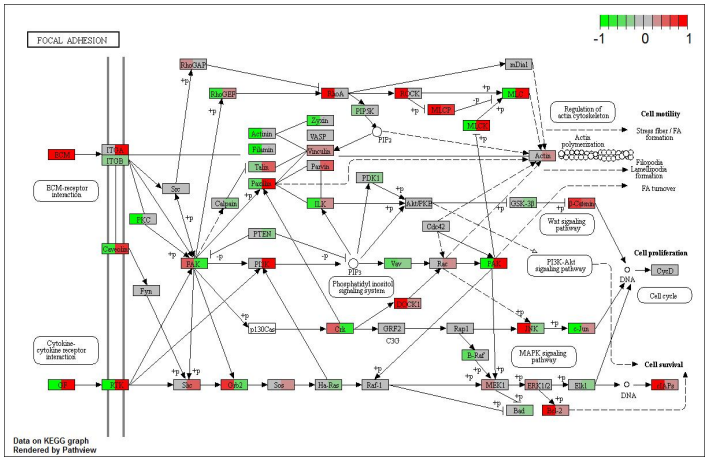

Figure 5 focal adhesion pathway of CYP24A1

The focal adhesion pathway showed ECM receptor interaction,MLCP,Beta-catenin have strong signal among this pathway.

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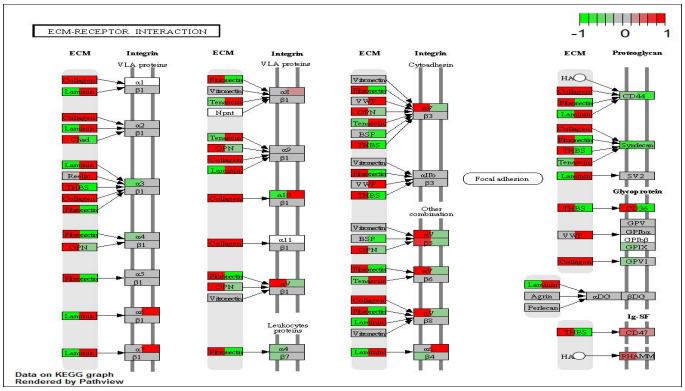

Figure 6 ECM receptor interaction pathway of CYP24A1

The ECM receptor interaction pathway indicate collagen,CD47,RHAMM showed strong signal compared with other genes.

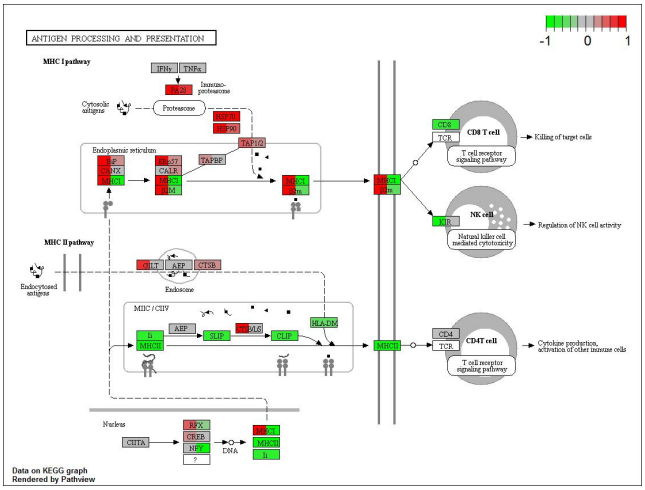

Figure7 Antigen process of CYP24A1

Related genes also showed antigen process of CYP24A1 having strong signal among other process.Antigen process and presentation reveals PA28,HS70,HSP90,BaP,ERp57,TAPBP are signal pathway genes which proved to be significant in this process.

4. Role of vitamin D3 : Microarray analysis of GSE55810 dataset

GSE55810 dataset using cells from human colon cancer.Cell lines were cultured either alone, with Vitamin D3, with THP1 macrophages, or with THP1 macrophages and Vitamin D3(Carvalho *et al.*, 2018).

All the selected study groups can be separate as series number 1 to 8, which are HCT116_VitD3,HCT116_alone,HCT116_THP1_coculture_VitD3,HCT116_THP1_coculture,Hke3_alone, Hke3_VitD3,Hke3_THP1_coculture_VitD3,Hke3_THP1_coculture,THP1_alone, THP1_VitD3,specifically.

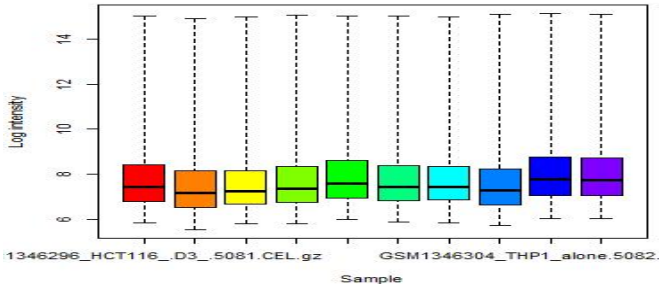

Figure 8. rainbow plot of GSE55810

ISSN:2790-1688 DOI: 10.56028/aetr.4.1.411.2023

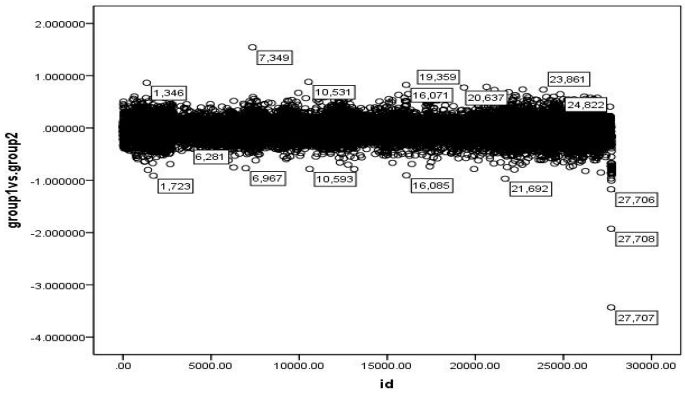

Figure 9. Scatter plot of mean difference between HCT116_VitD3 and HCT116_alone

After normalization of dataset gse55810, Welch Two Sample t-test was performed to find the difference between groups, ignoring the probe_id. The results showed there is statistical difference between group2 and group1(t=2.7089,p=0.006752). it illustrates vitamin D3 has effects compared with hct116 alone. On the other hand, t statistical significance has been found in goup3(HCT116_THP1_coculture_VitD3)andgroup2(HCT116_alone)(t=-3.1678,p=0.001563).

There is no statistical significance between group1(HCT116_VitD3) and group3(HCT116_THP1_coculture_VitD3)(t=0.46901,p=0.6391),group3(HCT116_THP1_coculture_VitD3) and group4(HCT116_THP1_coculture)(t=-1.2091,p=0.2266), after correction of P value, there is also no statistical significance between group2 and group4 (t=1.9707,p=0.04876),or the difference still need to be validated. No statistical significance has been found in four groups of Hke3_alone, Hke3_VitD3, Hke3_THP1_coculture_VitD3, Hke3_THP1_coculture,(all p value>0.17)There is no statistical difference between THP1_alone group and THP1_VitD3 group.(p=0.7096).

However, after gene list filtered (criteria with fold change >=2), 27740 genes have been selected to analyze.T-test between groups showed statistical significance, which is quite different from the results above.

Table 4 T test between four groups

Groups	T(test value= 0)	df	Sig.(two-sided)	Mean.diff	95% CI	
Group12	-40.351	27739	.00001	-.0382	-.0400	-.0363
Group32	-22.339	27739	.00001	-.0353	-.0384	-.0322
Group42	-6.743	27739	.00001	-.0108	-.0139	-.0076

However, comparison between group 1 and group2 is selected specifically to analyze differentially expressed genes.Therefore, the scatter plot showed the most significant genes,which were labeled. （ figure 10 ） Gene name information partly searched from PLANdbAffy database(Table 5).

Table5 Gene name identified in comparison of group1 and group 2.(figure 4)

ID	Identifier	Gene name
1346	1557459_at	SNF1LK2
1723	1559391_s_at	B4GALT5
6281	204455_at	dystonin(DST)
6967	205569_at	lysosomal associated membrane protein 3(LAMP3)
7349	206504_at	cytochrome P450 family 24 subfamily A member 1(CYP24A1)
10531	212464_s_at	fibronectin 1(FN1)
10593	212531_at	lipocalin 2(LCN2)
16071	222073_at	collagen type IV alpha 3 chain(COL4A3)
16085	222108_at	adhesion molecule with Ig like domain 2(AMIGO2)
19359	226444_at	SLC39A10

ISSN:2790-1688 DOI: 10.56028/aetr.4.1.411.2023

20637	228175_at	solute carrier family 4 member 8(SLC4A8)
21692	230061_at	transmembrane 4 L six family member 18(TM4SF18)
23861	235704_at	LOC647174
24822	238491_at	unknown
27706	AFFX-HUMRGE/	unkown
27707	AFFX-HUMRGE/	unkown
27708	AFFX-HUMRGE/	unkown

Using DAVID database analyze the GO terms of genes.We found vitamin D3 participate in several functions of genes,such as basement membrane, response to wounding and so on.(Table 6)

Table 6 Gene ontology terms of genes found in group 1 VS. group2

Term	P-Value	Benjamini
basement membrane	3.4E-2	8.4E-1
positive regulation of gene expression	4.8E-3	3.4E-1
cell adhesion	1.4E-2	4.7E-1
response to wounding	2.6E-2	5.3E-1
extracellular matrix organization	7.9E-2	8.3E-1
integrin binding	5.7E-4	1.5E-2
protease binding	3.5E-2	3.7E-1
iron ion binding	5.3E-2	3.8E-1

5. Methods

This article using GEO dataset 12449 and GSE55810 analysis, in which description, t test, pathway analysis,DAVID database search were applied .

6. Discussion

The pathview analysis also shows CYP24A1,participating the ECM receptor interaction pathway, in which also indicate collagen,CD47,RHAMM have quite large weight compared with other genes. Meanwhile, the focal adhesion pathway shows ECM receptor interaction, MLCP, Beta-catenin have big significance.Finally, antigen process and presentation reveals PA28,HS70,HSP90,BaP,ERp57,TAPBP are signal pathway genes which proved to be significant in this process.

Vitamin D3 make a significance in several gene ontology process, such as basement membrane, response to wounding, positive regulation of gene expression, iron ion binding, integrin binding and so on. Study by Penna,G et.al showed that The vitamin D receptor agonist elocalcitol inhibits IL-8-dependent benign prostatic hyperplasia stromal cell proliferation and inflammatory response by targeting the RhoA/Rho kinase and NF-kappaB pathways(Wali et al., 1992). Study by Dorshkind K,et.al reveals that 1,25-Dihydroxyvitamin D3 inhibits myelopoiesis but not B-lymphopoiesis in long-term bone marrow cultures(Dorshkind et al., 1989).

It reveals that vitamin D3 can response to membrane exchange and correlate to iron bindings. Study by Wali,RK et.al showed that 1,25-dihydroxyvitamin D3 inhibits Na(+)-H+ exchange by stimulating membrane phosphoinositide turnover and increasing cytosolic calcium in CaCo-2 cells(Dorshkind et al., 1989b; Wali et al., 1992b).

The results also showed vitamin D3 correlate to several genes, such as (CYP24A1), collagen type IV alpha 3 chain(COL4A3), solute carrier family 4 member 8(SLC4A8), solute carrier family 4 member 8(SLC4A8), collagen type IV alpha 3 chain(COL4A3) and so on. Study by Enioutina,EY et.al indicate that TLR-induced local metabolism of vitamin D3 plays an important role in the diversification of adaptive immune responses(Enioutina et al., 2009).

It indicate these genes make a big role to help vitamin D3 to participate in the gene ontology process. Their correlation may give some conclusion that CYP24A1 and other genes can influence

ISSN:2790-1688 DOI: 10.56028/aetr.4.1.411.2023
the presence and effect of vitamin D3,while the inside mechanism is unclear. Study showed recombinant human cystatin C and E 64 dose dependently inhibited the mobilization of 45Ca and the release of 3H (from [3H]-proline-labelled bones) in mouse calvariae stimulated to resorb by parathyroid hormone (PTH) or 1,25(OH)2-vitamin D3(Thakur, 2019; Vupperla et al., 2018; Manna et al., 2018; Wang et al., 2018).

Butyric acid is the main source of energy for the intestinal mucosal cells, more than 70% of the energy required for the intestinal mucosa comes from the oxidation of butyrate(Molina et al., 2013).

Although studies have described the relationship between butyric acid and CYP-24A1, the distinction between vitamin D3 and CYP24A1 is not clear. The article discusses this issue in detail. The analysis found that vitamin D3 is mainly involved in the membrane process and ion exchange, while cyp-24a1 has a positive regulatory effect on the inflammatory response.

Study by Schmiedlin-Ren et.al found that expression of enzymatically active CYP3A4 by Caco-2 cells grown on extracellular matrix-coated permeable supports in the presence of 1alpha,25-dihydroxyvitamin D3(Schmiedlin-Ren et al., 1997).

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