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內文：

Introduction

Aimed to illuminate the molecular regulation of angiogenesis in this SCC tumor entity in order to demask novel markers of prognosis or therapeutic approach

1. Angiopoietin 1 (ANGPT1): plays a significant role in endothelial cell survival and vascular maturation.
2. ANGPT2: acts as ANGPT1-Tie antagonist and exerts anti-angiogenic effects in several tumour entities. In OSCC, ANGPT2 overexpression comes along with increased malignancy and poor prognosis
3. VEGF(angiogenic cytokine), ANGPT2 induces apoptosis in endothelial cells in the absence of VEGF. The synergistic effect of VEGF and ANGPT2 then obviously results in tumour angiogenesis and poor prognosis.
4. Ephrin B2 (EFNB2) is a member of the receptor protein-tyrosin kinase family and is involved in many developmental processes; all members of the ephrin-B family are transmembrane proteins.

Material and methods

Angiogenic genes of 83 cancer samples were established by comparison to 30 samples of healthy oral mucosa with microarray technique. Immunohistochemistry (IHC) was performed to trace the signalling cascade from gene to protein level.

1. Patient data
   - 甲、 2009~2012
   - 乙、 Over 18 years old
2. RNA extraction and microarray assay
3. Immunohistochemistry
   - 甲、 IRS (immunoreactive score): multiplication of percentage of positive cells (PP) and staining intensity (SI) ranging from 0 to 12. Definition of PP score: PP = 0 no staining, PP = 1 staining in less than 10 % of cells, PP = 2 staining in 10 to 50 % of cells, PP = 3 staining in 50 to 80 % of cells, and PP = 4 staining in more than 80 % of cells. Definition of SI score: SI = 1 no staining, SI = 2 weak staining, SI = 3 moderate staining, and SI = strong staining.
4. Statistical analysis
   - 甲、 IRS score
   - 乙、 UICC(國際抗癌協會) stage and grading\
   - 丙、 ANOVA (方差分析)

Result

1. Expression factors of these genes increase with tumour size and grading (Tables 2 and 4)
2. The expression of PECAM1/CD31 correlated negatively with tumour size and grading

(Tables 1 and 3).

3. VEGF and EFNB2 showed a contrary gene expression in small and larger tumours with lymphatic spread. The expression increase in smaller tumours and decrease in larger tumours in contrast to tumours without lymphatic spread (Table 2).
4. In poorly differentiated tumours a higher gene expression of VEGF, EFNB2 and ANGPT2 was observed in tumours with lymphatic spread.
5. In moderately differentiated tumours, gene expression of VEGF and ANGPT2 were higher in tumours without lymphatic spread; only EBFNB2 showed here a higher gene expression (Table 4).

Table 1 Clinicopathological features of included OSCC samples

Localisation		TMN							
	Number (%)	T	Number (%)	N	Number (%)	G	Number (%)	UICC	Number (%)
Mouth floor	23 (27.7)	T1 + T2	56 (67)	N-	53 (64)	G1	2 (2.4)	I	15 (18)
Alveolar ridge	22 (25.4)	T3+ T4	27 (33)	N+	30 (36)	G2	67 (80.7)	II	22 (27)
Tongue	20 (24.0)					G3	14 (16.9)	III	9 (11)
Buccal plain	8 (9.6)							IV	37 (44)
Lip	5 (6.0)								
Palate	3 (3.6)								

Table 2 Expression factors of angiogenesis-related genes and IRS score related to T and N in OSCC samples

Gene/Protein	T1 + T2				T3 + T4			
	all (n = 56)	IRS score (n = 10)	N- (n = 39)	N+ (n = 17)	all (n = 27)	IRS score (n = 4)	N- (n = 14)	N+ (n = 13)
VEGF (NM_001025366)	4.13 ± 2.70 (53)	5.8	4.10 ± 2.40 (37)	4.91 ± 4.15 (16)	4.81 ± 2.32 (27)	7.0	5.32 ± 2.68 (14)	4.25 ± 1.72 (13)
PECAM1 (NM_000442)	0.76 ± 0.40 (41)	0	0.55 ± 0.20 (28)	0.58 ± 0.16 (13)	0.52 ± 0.18 (24)	0	0.49 ± 0.21 (13)	0.54 ± 0.14 (11)
EFNB2 (NM_004093)	2.24 ± 1.08 (46)	4.0	2.06 ± 0.90 (32)	2.56 ± 1.25 (14)	2.39 ± 1.12 (24)	5.5	2.4 0 ± 1.04 (13)	2.39 ± 1.22 (11)
ANGPT1 (NM_001146)	0.51 ± 0.32 (51)	1.2	0.41 ± 0.32 (35)	0.33 ± 0.20 (16)	0.29 ± 0.16 (27)	1.5	0.27 ± 0.15 (14)	0.33 ± 0.18 (13)
ANGPT2 (NM_001147)	3.43 ± 1.73 (52)	5.2	3.52 ± 1.73 (35)	3.51 ± 1.08 (17)	3.91 ± 1.92 (25)	5.8	4.40 ± 1.81 (13)	3.39 ± 1.99 (12)

Expression factors were experimentally determined in relation to a healthy oral mucosa pool with an expression factor of 1; displayed is the median; ± means Standard deviation; number of tumour samples in groups are in brackets

Table 3 Expression factors of angiogenesis-related genes and IRS score related to UICC in OSCC samples

Gene/Protein	UICC I (n = 15)		UICC II (n = 22)		UICC III (n = 9)		UICC IV (n = 37)	
	all	IRS score (n = 4)	all	IRS score (n = 5)	all	IRS score (n = 2)	all	IRS score (n = 3)
VEGF (NM_001025366)	3.21 ± 1.70 (14)	5.0	4.73 ± 2.59 (22)	6.4	2.89 ± 1.2 (8)	6.0	5.21 ± 3.3 (37)	7.3
PECAM1 (NM_000442)	0.63 ± 0.18 (9)	0.0	0.48 ± 0.17 (18)	0.0	0.48 ± 0.19 (7)	0.0	0.54 ± 0.17 (31)	0.0
EFNB2 (NM_004093)	1.48 ± 0.48 (11)	0.5	2.22 ± 0.93 (22)	6.0	1.60 ± 0.36 (7)	4.0	2.89 ± 1.48 (31)	7.3
ANGPT1 (NM_001146)	0.47 ± 0.18 (12)	1.5	0.31 ± 0.19 (22)	1.2	0.33 ± 0.25 (9)	0.0	0.33 ± 0.17 (36)	2.0
ANGPT2 (NM_001147)	3.81 ± 2.42 (13)	6.5	3.28 ± 1.46 (21)	4.0	2.96 ± 1.12 (9)	6.0	3.77 ± 1.69 (36)	5.7

Expression factors were experimentally determined in relation to a healthy oral mucosa pool with an expression factor of 1; displayed is the median; ± means Standard deviation; number of tumour samples in groups are in brackets

Table 4 Expression factors of angiogenesis-related genes and IRS score related to G and N in OSCC samples

Gene/Protein	G1 (n = 2)				G2 (n = 67)				G3 (n = 14)			
	all	IRS score (n = 2)	N- (n = 1)	N+ (n = 1)	all	IRS score (n = 9)	N- (n = 44)	N+ (n = 23)	all	IRS score (n = 3)	N- (n = 8)	N+ (n = 6)
VEGF (NM_001025366)	4.99 ± 3.90 (2)	8	1.09	8.9	4.22 ± 2.46 (65)	5.8	4.64 ± 2.65 (43)	3.47 ± 1.81 (23)	5.62 ± 3.92 (14)	6	3.1 ± 1.25 (8)	8.29 ± 6.44 (6)
PECAM1 (NM_000442)	0.98 ± 0.11 (2)	0	0.87	1.09	0.57 ± 0.18 (54)	0	0.55 ± 0.18 (36)	0.59 ± 0.16 (18)	0.36 ± 0.15 (12)	0	0.24 ± 0.09 (5)	0.47 ± 0.09 (6)
EFNB2 (NM_004093)	1.08 ± 0.69 (2)	4	0.41	1.76	2.36 ± 0.66 (55)	6.7	2.17 ± 0.92 (38)	2.72 ± 1.66 (19)	2.69 ± 1.08 (12)	1.3	2.13 ± 0.9 (5)	3.16 ± 1.18 (6)
ANGPT1 (NM_001146)	1.13 ± 0.17 (2)	6	1.3	0.96	0.34 ± 0.19 (64)	0.7	0.33 ± 0.19 (41)	0.36 ± 0.19 (23)	0.28 ± 0.17 (14)	0	0.36 ± 0.25 (8)	0.2 ± 0.07 (6)
ANGPT2 (NM_001147)	2.55 ± 1.68 (2)	4.5	0.87	4.23	3.53 ± 1.65 (64)	5.9	3.86 ± 1.89 (41)	2.94 ± 1.20 (23)	3.39 ± 1.82 (13)	6.7	1.81 ± 0.45 (6)	5.25 ± 1.47 (6)

Expression factors were experimentally determined in relation to a healthy oral mucosa pool with an expression factor of 1; displayed is the median; ± means Standard deviation; number of tumour samples in groups are in brackets

Table 5 Gene expression ratio of ANGPT1 to ANGPT2

Ratio	T1 + T2	T3 + T4
Ang1/Ang2 all	0.23 ± 0.32	0.15 ± 0.17
Ang1/Ang2 (N-)	0.25 ± 0.37	0.08 ± 0.08
Ang1/Ang2 (N+)	0.16 ± 0.15	0.22 ± 0.18

Ratio in healthy pool 1.0; ± means Standard deviation

Discussion

Tumour-related neo-angiogenesis is an important prerogative for tumour growth and spread.

In colon carcinoma, antiangiogenic therapy has proven its effect. Our data demonstrate in malignant

tumours a significantly higher expression of VEGF, ANGPT2 and EFNB2 at both, gene and protein levels, in malignant tumours when compared to normal oral mucosa. ANGPT1 and PECAM1/CD31 were regulated reversely when compared to ANGPT2. In conclusion, the analysis of patient data led us to the suggestion that the progressive lack of ANGPT1 from G1 to G3 tumours comes with reduced vessel ripening which is marked immunohistochemically by weak CD31 staining.

1. Descend of the Ang1/Ang2 ratio correlates with pronounced vascularization and poor prognosis
2. Low Ang1/Ang2 ratio was observed on larger tumour size
3. Expression of ANGPT1 was associated with lymphatic metastasis
4. ANGPT2 seems to play an outstanding role in the development of metastatic spread and thus must be considered as a prospective therapeutic target for attacking progressive disease
5. EFNB2 seems to be a relevant mediator not only in vessel differentiation but also in vascular sprouting

Conclusion

Two major findings of our investigation are on the one hand the distinctive expression pattern of the angiogenic mitogens and on the other hand the characteristic inversely correlated Ang1/ Ang2 expression ratio. Starting point for future investigations would be the analysis of vessel maturation in malignant tissue as well as the relevanceand reliability of the angiopoietin ratio as a prediction factor of tumour progression and prognosis.

答案	題目
(A)	在臨床組織學上,跟 keratocanthoma相似的為? (A)分化良好的SCC (B)中度分化的SCC (C)低度分化的SCC (D)無分化的SCC
(C)	列何者最可能發生惡性變化 (A)輕度上皮變異 (B) 中度上皮變異 (C) 重度上皮變異 (D)上皮增生