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Investigation of EGFR copy-number variations in skin cancers

Indagine sulle varianti di copia-numero di EGFR nel cancro della pelle

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Summary

Aim. **The epidermal growth factor receptor (EGFR) family members play a considerable rôle in carcinogenesis. Copy number variations (CNVs) have been discovered to have phenotypic consequences and to be associated with various types of cancer. CNVs of EGFR were associated with cancer pathogenesis in recent studies. However, few studies were performed in skin cancer. In this study, we aim to examine the CNVs of EGFR in skin samples.** ***Materials and methods.*** **A total of 195 paired-samples including basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and actinic keratosis (AK) were included. Real-time PCR was used for the quantification of EGFR copy numbers.** ***Results.*** **CNVs of EGFR showed statistical differences between cancer samples (both SCC and BCC) and normal tissues ($p<0.05$). Association analysis showed that the frequencies of EGFR's CNVs were correlated**

Riassunto

Obiettivo**. I membri della famiglia del Recettore del Fattore di Crescita dell'Epidermide (Epidermal Growth Factor Receptor, EGFR) svolgono un ruolo considerevole nella carcinogenesi. Si è dimostrato che le varianti nel numero di copie (Copy Number Variations, CNVs) avevano implicazioni fenotipiche ed erano associate a vari tipi di cancro. Le CNVs dell'EGFR sono state associate alla patogenesi del cancro in studi recenti. Tuttavia, pochi studi sono stati condotti sul cancro della pelle. In questo studio, ci proponiamo di esaminare le CNVs dell'EGFR in campioni di pelle.** ***Materiali e metodi.*** **Un totale di 195 campioni appaiati, tra cui il carcinoma a cellule basali (Basal Cell Carcinoma, BCC), il carcinoma a cellule squamose (Squamous Cell Carcinoma, SCC) e cheratosi actinica (Actinic Keratosis, AK), sono stati inclusi. La metodologia Real-Time PCR è stata utilizzata per la quantificazione del numero**

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with the severity of skin abnormalities (p=0.011). *Conclusions.* CNVs of EGFR are associated with SCC and BCC but not with AK. Eur. J. Oncol., 16 (1), 15-19, 2011

di copie di EGFR. *Risultati.* Le CNVs dell'EGFR hanno mostrato differenze statistiche tra campioni di cancro (sia SCC che BCC) e tessuti normali (p<0.05). Analisi di associazione hanno mostrato che le frequenze delle CNVs dell'EGFR sono state correlate con la gravità delle anomalie della pelle (p=0.011). *Conclusioni*. Le CNVs dell'EGFR sono associate a SCC e BCC, ma non a AK. Eur. J. Oncol., 16 (1), 15-19, 2011

Key words: **EGFR, copy-number variations (CNVs), basal cell carcinoma (BCC), squamous cell carcinoma (SCC), actinic keratosis (AK)**

Parole chiave: **EGFR, varianti del numero di copie (CNVs), carcinoma a cellule basali (BCC), carcinoma a cellule squamose (SCC), cheratosi actinica (AK).**

Introduction

The epidermal growth factor receptor (EGFR) family members play a considerable rôle in carcinogenesis through their involvement in proliferation, apoptosis, angiogenesis and metastasis (1). Furthermore, overexpression of EGFR has been correlated with more aggressive phenotypes and overall poor prognosis (2, 3). The predictive and prognostic value of EGFR amplification and overexpression in cancer has become a hot spot in the past few years, resulting in the development of numerous targeted therapies (1). The EGFR gene is located on the short arm of chromosome 7 (7p21), and molecular analysis searching for aberrations in the gene has also indicated that an increased EGFR copy number can be detected in Non–Small-Cell Lung Cancer (NSCLC), colorectal cancer and Squamous Cell Carcinoma of the Head and Neck (HNSCC) (4, 5). Several methods for detecting and determining EGFR gene copy number and dosage are available, including Fluorescence In Situ Hybridization (FISH) (6), Chromogenic In Situ Hybridization (CISH) (7) and Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) (8, 9).

Copy number variations (CNVs) were originally defined by the presence of variable numbers of copies of large, multi-kilobase genomic regions in the genomes of different individuals (10). However, recent high-resolution genome maps have revealed smaller CNVs among healthy humans (10, 11), thus extending the definition of CNVs to the length of regions being as short as several hundred bases. Several methodologies, such as the most commonly used array-based Comparative Genomic Hybridization (aCGH), were utilized for genome-wide CNV detection and genotyping. CNVs have been discovered to have phenotypic consequences and to be associated with various types of cancers over the past few years (12, 13). However, few CNVs studies were performed in skin malignancies.

Skin cancers are divided into melanoma and non-melanoma. Non-melanoma, which is about 20 times more common than melanoma, includes Basal Cell Carcinoma (BCC), Squamous Cell Carcinoma (SCC) and other types of skin cancer. Actinic Keratosis (AK) is considered the earliest stage in the development of skin cancer and shows the potential to progress to SCC. In our study, we examined the CNVs of EGFR in 195 paired-samples including BCC, SCC and AK.

Materials and methods

Control and patient samples

Surgically resected tumour tissues and adjacent normal tissues were collected from 67 Squamous Cell Carcinoma (SCC), 85 Basal Cell Carcinoma (BCC) and 43 Actinic Keratosis (AK) patients. The

study was approved by the ethical committee of Peking University Shenzhen Hospital. The individuals gave their written informed consent. The investigations were carried out according to the Declaration of Helsinki principles.

DNA extraction and quantification of copy numbers

Genomic DNA was isolated from the tissues using the Genomic DNA Extraction Kit (Innogent, Shenzhen, China) according to the manufacturer's instruction. Quantitative PCR was performed through BioRad Chromo4 Real-Time PCR system. Average copy numbers of RNAse P in normal candidates (copy numbers=2) were used as control (14). The copy numbers of EGFR were calculated by using the comparative C(T) method. Cut-off values of 0.33, 0.67 and 1.33 were used to define the copy numbers as 0, 1 and 2 respectively. The primers for RNAse P are: forward: 5'-AGA CTA GGG TCA GAA GCA A and reverse: 5'-CAT TTC ACT GAA TCC GTT C. The primers for EGFR are: forward: 5'- CTT TCG ATA CCC AGG ACC AA and reverse: 5'- ACT TCC TGG CTA GTC GGT GT. Statistical analysis was performed using chi-square test or Fisher exact test. Association analysis was performed using linear-by-linear association test. P values less than 0.05 were considered as statistically significant.

Results

Table 1 shows CNVs of EGFR in skin samples. A total of 195 paired-samples were examined. Statistical differences were observed in SCC and BCC samples as compared with the normal tissues ($p<0.05$). However, there was no significant difference between AK samples and controls ($p=0.306$). We also calculated the Odds Ratios (OR) values by combining the samples with >2 copies of DNA, and the amplified copy numbers of EGFR were found to increase the risk of SCC (OR=20.7, 95%CI 2.7-161.3) and BCC (OR=6.2, 95%CI 1.3-28.7), but not the risk of AK (OR=3.2, 95%CI 0.3-31.6).

The highest frequencies of EGFR CNVs were observed in SCC samples, while the lowest frequencies were observed in AK samples. Since SCC is known to be more malignant than BCC, while AK belongs to skin pre-malignancy, we then performed the association analysis among different types of abnormalities (Table 2). Statistical differences were observed for EGFR ($p=0.011$), indicating that the frequencies of CNVs may be correlated with the severity of skin abnormalities.

Discussion

CNVs have been clearly shown to have the potential to directly or indirectly influence a healthy indi-

Table 1 - CNVs of EGFR in skin samples*

Population	No.	Copy numbers			*p*	Copy numbers		*p*	OR (95% CI)
		=2	=3	>=4		=2	>2		
Total									
Cancer samples	152	122	25	5	1.17E-05	122	30	2.23E-06	9.1 (3.1-26.5)
Normal tissues	152	148	4	0		148	4		
SCC									
Cancer samples	67	51	13	3	4.98E-04	51	16	9.89E-05	20.7 (2.7-161.3)
Normal tissues	67	66	1	0		66	1		
BCC									
Cancer samples	85	74	9	2	0.031	74	11	0.009	6.2 (1.3-28.7)
Normal tissues	85	83	2	0		83	2		
AK									
Abnormal tissues	43	40	3	0	0.306	40	3	0.306	3.2 (0.3-31.6)
Normal tissues	43	42	1	0		42	1		

* CNVs=Copy Number Variants; SCC=Squamous Cell Carcinoma; BCC=Basal Cell Carcinoma; AK=Actinic Keratosis
OR=Odds Ratios; 95% CI=95% Confidence Interval

Table 2 - Association analysis of CNVs of EGFR in SCC, BCC and AK samples*

Population	No.	Copy numbers			*p*	Copy numbers		*p*
		=2	=3	>=4		=2	>2	
SCC	67	51	13	3		51	16	
BCC	85	74	9	2	0.011	74	11	0.013
AK	43	40	3	0		40	3	

* CNVs=Copy Number Variants; SCC=Squamous Cell Carcinoma; BCC=Basal Cell Carcinoma; AK=Actinic Keratosis OR=Odds Ratios; 95% CI=95% Confidence Interval

vidual's susceptibility to cancer, for example by varying the gene dosage of tumour suppressors or oncogenes (15, 16). It is suggested that the genes present in very small regions of CNVs are excellent candidates for evaluation in cancer pathogenesis. Examination of the CNVs for such genes helps to understand the functional consequences of these CNVs. Previous studies have shown that CNVs of EGFR were associated with cancer pathogenesis (4, 5). However, in skin cancer, few such studies were performed yet. In our study, we found that CNVs of EGFR are associated with SCC and BCC but not AK. The significance of these findings was supported by some previous studies. It was demonstrated that high EGFR expression by immunohistochemical staining (IHC) was associated with poor survival of HNSCC (17, 18). Evaluation of EGFR gene copy numbers by Fluorescence In Situ Hybridization (FISH) confirmed the amplification of this gene in cancer samples (19, 20). In addition, in a comparative genomic hybridization analysis, oral squamous cell carcinomas showed a gain of chromosome 7p including band p12, and the patients with the 7p genomic gain had a higher rate of relapse and worse survival (21). It is well known that activation of the EGFR pathway is associated with radiation resistance, and that EGFR blockade with cetuximab can modulate the radiation effect in head and neck cancer (22, 23). Several EGFR inhibitors, including cetuximab, have been studied in recurrent or metastatic HNSCC patients and found to have modest response rates (24, 25). The results from our study further extended the understanding of EGFR in human skin malignancies. However, the functional consequences of such CNVs need to be extensively investigated in the future.

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