Egfr amplification specific gene expression in phyllodes tumours of the breast

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Abstract. Background: Recently, we were able to show that amplifications of the epidermal growth factor receptor (egfr) gene and the overexpression of EGFR were associated with the initiation and progression of phyllodes tumours. Methods: In order to gain further insights into regulation mechanisms associated with egfr amplifications and EGFR expression in phyllodes tumours, we performed global gene expression analysis (Affymetrix A133.2) on a series of 10 phyllodes tumours, of these three with and seven without amplifications of an important regulatory repeat in intron 1 of egfr (CA-SSR I). The results were verified and extended by means of immunohistochemistry using the tissue microarray method on an extensively characterized series of 58 phyllodes tumours with antibodies against caveolin-1, eps15, EGF, TGF-α, pErk, pAkt and mdm2. Results: We were able to show that the presence of egfr CA-SSR I amplifications in phyllodes tumours was associated with 230 differentially expressed genes. Caveolin-1 and eps15, involved in EGFR turnover and signalling, were regulated differentially on the RNA and protein level proportionally to egfr gene dosage. Further immunohistochemical analysis revealed that the expression of caveolin-1 and eps15 were also significantly correlated with the expression of pAkt (p < 0.05), pERK (p < 0.05), mdm2 (p < 0.01) and EGF (p < 0.001 for caveolin-1). Eps15 and pERK were further associated with tumour grade (p < 0.01 and p < 0.001, respectively). Conclusion: Our results show that amplifications within regulatory sequences of egfr are associated with the expression of eps15 and caveolin-1, indicating an increased turnover of EGFR. The interplay between EGFR and caveolin-1, eps15, pAkt, mdm2 and pERK therefore seems to present a major molecular pathway in carcinogenesis and progression of breast phyllodes tumours. Keywords: Phyllodes tumours, egfr, caveolin-1, eps15

1. Introduction

Phyllodes tumours (PT) of the breast are rare biphasic tumours which account for less than 1% of all breast tumours. Their pathogenesis and underlying genetic alterations have been poorly understood. Recently, we were able to show that overexpression of the Epidermal Growth Factor Receptor (EGFR) was correlated with the progression of these tumours. Furthermore, EGFR overexpression was strongly associated with whole gene amplifications of egfr gene as well as with amplifications within intron 1 of egfr, the latter containing important regulatory sequences for the transcription of the gene (CA-SSR I) [9]. These results were largely in line with previous results from the literature for other proteins and provided for the first time a more comprehensive idea about egfr biology of these tumours [2–4]. Since genetic alterations of egfr, which are associated with overexpression of its protein, are among the first recurrent molecular alterations described in phyllodes tumours, amplifications of egfr may play a role in the initiation and progression of a large subset of phyllodes tumours. Amplifications of CA-SSR I have also repeatedly been described in invasive breast cancer and were similarly correlated with EGFR overexpression [3,31]. Nevertheless, the affected intracellular pathways associated with these mutations could not be defined so far.
In this study we aimed to define intracellular relationships and possible pathways downstream of egfr by means of global gene chip analysis in a series of PT, characterized for their egfr CA-SSR I amplification status. This was followed by the immunohistochemical verification of candidate genes in a tissue microarray approach. Our findings provide more insight into putative regulation pathways associated with egfr amplifications in PT of the breast and stress the importance of egfr as a driving force in the initiation and progression of these intriguing tumours.

2. Materials and methods

2.1. Materials

Fresh frozen tumour tissue of 10 PT taken from the archives of the Departments of Pathology, Universities of Muenster and Utrecht, were used for gene chip analysis. These consisted of 5 benign, 3 borderline and 2 malignant tumours according to the criteria of Moffat et al. [21]. Scoring of tumour grade as well as immunohistochemical scoring was performed independently by two pathologists (C.K. and H.B.). Cases with disagreement were scored jointly using a double-headed microscope.

All 10 tumours were evaluated for the expression of EGFR on fresh frozen tissue, since sufficient paraffin-embedded tumour material was not for all available anymore. All ten tumours showed positive EGFR immunoreactivity within the stromal and the myoepithelium compartments. The glandular epithelium was consistently negative. Paraffin blocks of 58 PT of the female breast were retrieved from the archives of the Institute of Pathology, University of Muenster, the Institute of Pathology in Osnabrueck and the Institutes of Pathology in Koeln-Rodenkirchen and Limburg, Germany. The use of tumour tissue was also approved by the local ethical committee.

All tumours were characterized for their egfr gene dosage status by means of quantitative real-time PCR and FISH analysis. In addition, expression of EGFR, p16, p21, p27, p53, c-myc, Cyclin A, Cyclin D1, Cyclin E, c-kit and Ki-67 was studied by immunohistochemistry as previously described [8,9,35].

2.2. mRNA expression microarray

Total RNA (tRNA) was isolated from each of the samples using the RNeasy Mini Kit (Qiagen GmbH) according to the manufacturer’s instructions. Quantity and purity (260/280 nm ratio) was determined using a BioPhotometer (Eppendorf, Germany). Quality control of the isolated tRNA was done on an automated electrophoresis system (BIORAD Experion Bioanalyser, USA) using RNA StdSens Chips. With this LabChip microfluidic system, the 28S/18S ratio was calculated and only tRNA without degraded amounts of tRNA was used in this study.

1 μg of RNA per sample was introduced in the process. If the concentration of the RNA was lower than 1 μg/8 μl an enrichment step was applied (Microcon filter, Millipore). All further steps were strictly based on the Affymetrix protocol and the Affymetrix kits. The cDNA synthesis was done with the ‘One-Cycle’ process. cDNA clean up, in vitro transcription and cRNA clean up were performed with the respective kits/procedures provided by Affymetrix. The quantity of the biotin-labeled cRNA was checked by measuring the 260/320 nm ratio. Hybridization and scanning were performed individually for each of the samples on the basis of the oligonucleotide microarray HG U133Av2 (22277 probe sets, Affymetrix) according to the manufacturer’s protocols.

2.3. Microarray analysis

GeneChip® Operating Software (GCOS, Affymetrix) version 1.4 was used to evaluate the raw data (for GCOS algorithms see Affymetrix: Statistical Algorithms Description Document – sadd_whitepaper.pdf). Signal values were exported from GCOS and imported into the statistics software package S-Plus 6.2. In this environment the different experiments were scaled to the same interquartile range and median. The resulting new experimental reference parameters were strictly comparable concerning the median and the spread of the data. The significant gene expression differences between the defined experimental groups were evaluated using t-test (two-sample, two-sided, variance equal, alpha < 0.05). The additional filter criteria to call a gene “differentially expressed” were an F test p-value < 0.05, a fold change value > 1.5 and a difference of the group means > 10.
2.4. Tissue microarray

A tissue microarray of 58 phyllodes tumours of the breast with six cores of 0.6 mm in diameter were punched out of the donor block according to standard protocols using a dedicated TMA instrument (Beecher Instruments, Silver Spring, Maryland, USA) [13,23] and transferred to a recipient block as previously described [32].

2.5. Immunohistochemistry

Table 1 shows the sources of the antibodies, dilutions, and antigen retrieval methods applied. For all antibodies, endogenous peroxidase activity was blocked for 30 minutes in a methanol solution containing 0.3% hydrogen peroxide after deparaffination and rehydration. After antigen retrieval, a cooling off period of 30 minutes preceded the incubation (overnight at 4°C) with the primary antibody. Before the slides were mounted all sections were dehydrated in alcohol and xylene. Only the immunohistochemical results of the stromal part (dominating phyllodes tumours) were used for detailed analysis. Staining intensities were graded semiquantitatively from 0 (weak) to 3 (strong) using positive controls recommended by the antibody supplier.

2.6. Statistical analysis

Statistical analysis and tests were performed with SPSS Version 11.5.1. Correlations between EGFR expression, amplification and clinicopathological features were tested with cross tables applying Chi-square, and correlation analysis was performed according to Kendall (Tau b).

3. Results

3.1. Gene chip analysis

From all tumours sufficient high quality RNA could be isolated. All tumours presented unaltered copy numbers for the egfr gene by means of FISH analyses. Three tumours (1 malignant; 2 benign) displayed amplifications within intron 1 of egfr, seven tumours (1 malignant; 3 borderline; 3 benign) revealed a normal gene dosage for this specific locus using quantitative real time PCR. 213 genes were upregulated and 17 genes were downregulated in PT with CA-SSR I amplifications at a significance level of 0.05 with multiple t-testing (Table 2). The fold change value was set to a moderate level of greater 1.5 fold. The resulting number of differentially regulated genes is in accordance with the applied constraints. Numerous genes were found involved either in nucleic acid metabolism (n = 42) or signal transduction (n = 40). Further, twelve genes were found to be directly involved in receptor tyrosine kinase signalling pathways as shown in Table 3.

3.2. Immunohistochemical verification of differentially regulated genes

Due to lack of sufficient paraffin embedded material the samples used for expression profiling were not included in the tissue microarray. In the used cases amplifications of CA-SSR I were found in 15/38 benign, 2/9 borderline and 6/8 malignant phyllodes tumours. Differential regulation of genes, all known to be associated with EGFR signalling turnover and regulation, were verified on the protein level: caveolin-1, eps15, pAkt, mdm2, pERK, EGF and TGF-α. Representative images are shown in Fig. 1. Overviews about all significant correlations and about staining frequencies are given in Tables 4 and 5, respectively.

| Antibody | Source | Clone | Pretreatment | Dilution |
|----------|--------|-------|--------------|----------|
| Caveolin-1 | BD Biosciences | 2297 | 30′ steamer citrate buffer pH 6.0 | 1:200 |
| eps15 | Santa Cruz | - | - | 1:200 |
| pAkt | Cell Signaling Technology | rabbit polyclonal | 30′ steamer citrate buffer pH 6.0 | 1:200 |
| pERK | Cell Signaling Technology | E 10 | 30′ steamer citrate buffer pH 6.0 | 1:100 |
| EGF | Acris | rabbit polyclonal | 30′ steamer TRS pH 9.0 | 1:20 |
| TGF-α | Zytomed | MF9 +TG86 | 30′ steamer citrate buffer pH 6.0 | 1:15 |
| mdm2 | Zytomed | IF 2 | 30′ steamer TRS pH 9.0 | 1:50 |
### Table 2

List of candidate genes with upregulation and downregulation (italics) in phyllodes tumours with amplifications including sequences of intron 1 of the *egfr* gene

| Amino acid metabolism | GLUL; TDO2; MGC15523 |
|-----------------------|----------------------|
| Apoptosis             | BNIP3; CLU; KIAA0367; CASP4; GADD45A |
| Carbohydrate metabolism | FLJ21865; PPP1R3C; ENO2; ME1; A4GALT; MAN2C1; SLC2A5 |
| Cell adhesion         | ITGB1BP1; LRRC15; PPFBP1 |
| Cell cycle            | 11-Sep; GAS2; RECK; TBCD |
| *CDC2*; *CETN2*       | |
| Cell structure, proliferation and differentiation | AH11; ANK2; BST2; CDC42BPA; COL13A1; COL16A1; COL6A2; IFI44; NEF3; NEFL; PTDSR; SGCD |
| *ACTB*                | |
| Developmental processes | DVL3; IFRD1; LPIN1; MATN2; MBNL1; MEF2A; PKD2 |
| *ANXA2P2*             | |
| Immunity and defense  | C1QTNF3; CFH; CFH // CFHL1; NCF2; ORM1; PACS2; TNFAIP2 |
| Intracellular protein traffic | EPS15; EXOC7; GD11; ITSN2; LRP1; MRC2; MYO1D; PHLDB1; PPIG; TGOLN2 |
| Lipid, fatty acid and steroid metabolism | APOL1; LSS; PIK4CA // LOC220 |
| *PSAP*                | |
| Nucleoside, nucleotide and nucleic acid metabolism | ADBR1; ADNP; AKAP9L; ARI1GEF10; BCL6; CNOT4; DICER1; DPYD; ERCC3; GATA1; GUK1; HIST1H2BD; IRF3; KIAA0476; KIAA1466; LRRFIP1; MBD4; PLG1; PTBP1; PTG1; RBM6; RBPM5; SAFB2; SF1; SFRS11; SHOX2; SIN3B; SMARCC2; SMARC3; SOX3; SRRM1; SYNE1; THRAP2; THRAP5; TRA2A; TWIST1; VGLL4; ZBTB1; ZIC1; ZNF160; ZNF202; ZNF500; ZNF516 |
| SET; *MGMT*; *RNG7T*; *CPSF5* | |
| Oncogenesis            | EPB41L1; MAGEA9; TPD52L2 |
| Protein metabolism and modification | AAK1; CSNK1G2; CTSB; FASTK; HSPB7; IMP-3; LONP; MAST2; MKNK1; MMP14; NPEPL1; PAM; PTK2B; SERPINE1; SERPIN11; TAOK3; TTC15; TYK2; UBE2D2; USP53; WNK1 |
| *MRPS16*; *RPL5*; *YME1L1*; *OA2* | |
| Protein targeting and localization | AKAP10; GOLGA2; PDE4DIP; PPFBP1 // LOC44 |
| Signal transduction    | ADCY7; APBB3; C5R1; CAV1; CELSR1; CLEC7A; DDEF1; DDEF2; DDEF1L1; DLC1; DMPK; Epor; FGFR1; FN1; GNA11; GPR153; IL6; LAMA2; LAT; LEFTY2; LRP5; MAP2K3; MAPK8IP3; MERTK; MTSS1; PIK3R1; PKD1; PPAP2A; PRELP; RASAL2; RFNG; RGS1; ROCK2; SH2B; SHC1; SHC2; SN; STK22; TFRSF25; TSPAN8 |
| Transport              | ABCF2; ATP2B4; KCNAB1; MSCP; SCN4A; SFXN3; SLC1A3 |
Table 2 (Continued)

Biological process unclassified
AB13BP; BGN; DCCAG33; CHCHD7; DKEFZP434C171; DYM; EBP1L1; EHD2; RAB42; FAM13A1; FLJ10534; FLJ12788; FLJ13769; FLJ20507; FLJ34663; GOLPH4; IDS; KIAA0100; KIAA0265; KIAA0708; KIAA1536; KIAA1659; LOC399491; LOC51326; MUM1; PLEKHA5; RCK; REC14; RSAD1; SLC26A10; TAZ; TMEM45A; ZCWPW1; ZGPAT; ZNF638

C21orf33; FLJ10156; HBXIP; SPFH1

For all genes either a known or an inferred biological function was identified from Panther (www.pantherdb.org/genes), NETAFFX (www.affymetrix.com/analysis/netaffx/index.affx), OMIM (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) and GO (www.geneontology.org) nomenclature.

Table 3

List of differentially expressed candidate genes involved in signal transduction with association to the EGFR pathway

| Gene symbol | Chromosomal localization | Biological function                  |
|-------------|--------------------------|-------------------------------------|
| LAT         | 16p12.1                  | Ras protein signal transduction     |
| RASAL2      | 12q23–q24                |                                     |
| CSR1        | 19q13.3–q13.4            | MAPK cascade                        |
| MAP2K3      | 17q11.2                  |                                     |
| FGFR1       | 8p11.2–p11.1             | MAPKKK cascade                      |
| IL6R        | 1q21                     |                                     |
| SH2B        | 16p12.1                  |                                     |
| SHC1        | 1q21                     |                                     |
| SHC2        | 1q21                     |                                     |
| TNFRSF25    | 6p21.1–12.2              |                                     |
| CAV1        | 7q31.1                   | Receptor mediated endocytosis       |
| EPS15       | 1p32                     |                                     |

All shown genes were upregulated in phyllodes tumours with amplifications including sequences of intron 1 of the egfr gene. For all genes either a known or an inferred biological function was identified from Panther, NETAFFX, OMIM and GO nomenclature.

**Caveolin-1** was expressed in the cytoplasm of 9/53 (17%) PT. Staining was observed in both stromal and epithelial components. Caveolin-1 expression was positively correlated with the CA-SSR I gene dosage \((p < 0.05)\), pAkt \((p < 0.01)\), EGF \((p < 0.001)\), mdm2 \((p < 0.01)\), pERK \((p < 0.05)\), pAkt \((p < 0.05)\), pERK \((p < 0.05)\), pAkt \((p < 0.05)\), Cyclin A \((p < 0.05)\) and Cyclin E \((p < 0.001)\).

**eps15** showed a positive cytoplasmic immunoreactivity in 10/51 (20%) PT. Staining was observed in both stromal and epithelial components. Eps15 was significantly correlated with pAkt \((p < 0.05)\), mdm2 \((p < 0.01)\), pERK \((p < 0.01)\), c-kit \((p < 0.05)\), p53 \((p < 0.01)\), Ki-67 \((p < 0.01)\) and Cyclin A \((p < 0.05)\). A borderline statistical significance could be shown for the association between eps15 and CA-SSR I gene dosage \((p = 0.05)\).

**pAKt** revealed a positive immunostaining in the cytoplasm \((5/55; 9\%)\) of stromal and epithelial cells. Cytoplasmic staining of pAkt was positively associated with caveolin-1 and eps15 for EGF \((p < 0.01)\), TGF-\(\alpha\) \((p < 0.01)\), and pERK \((p < 0.01)\). Nuclear staining correlated significantly with EGFR expression \((p < 0.01)\). A trend towards statistical significance could be seen for the correlation with mdm2 \((p = 0.05)\).

**pERK** expression was sparse and could be seen in epithelium and stroma of 4 out of 56 cases (7%). Myoepithelial cells were mostly positive. Besides the above mentioned correlations, pERK expression was associated with TGF-\(\alpha\) \((p < 0.01)\), c-kit \((p < 0.05)\), p53 \((p < 0.001)\), Ki-67 \((p < 0.01)\) and tumour grade \((p < 0.001)\).

**EGF** was expressed in stromal cells of 10 out of 53 PT cases (19%), but was observed predominantly in the epithelial cell component. A significant positive correlation could be demonstrated for pAkt \((p < 0.05)\), TGF-\(\alpha\) \((p < 0.01)\), c-kit \((p < 0.05)\), Caveolin \((p = 0.001)\) and Cyclin E \((p < 0.05)\).

**TGF-\(\alpha\)** expression was observed predominantly in epithelial tumour cells. Cytoplasmic staining in stromal cells was seen in 11 out of 54 cases (20%). TGF-
α expression was further significantly associated with tumour grade ($p < 0.001$).

Nuclear mdm2 expression was seen in 11 of 54 cases (20%), predominantly in the stromal component. mdm2 expression in stromal cells was associated with expression of EGFR ($p < 0.05$), c-kit ($p < 0.01$), p53 ($p < 0.01$), Mib-1 ($p < 0.01$), p21 ($p < 0.001$), and Cyclin E ($p < 0.01$).

4. Discussion

Our own recently published study showed correlations between amplifications of egfr and EGFR overexpression in breast PT [9]. However, these results, in context with other reports, raised important questions and urged the need for an improved understanding of receptor tyrosine kinase regulated pathways in these tumours [26,27]. Unfortunately, at the present state and to the best of our knowledge, no cellular models like PT cell lines are available for detailed functional research. In order to circumvent these problems and to improve the knowledge about these tumours, we performed global gene expression analysis on a series of 10 phyllodes tumours with known status of egfr CA-SSR 1 amplifications. We are aware that the number of tumours might be too small to draw definite conclusions, but one has to take into account the low in-
Table 4
Overview about the significance of found immunohistochemical correlations

|        | pAKT | Cav-1 | eps15 | pERK | MDM2 | TGF-α | EGF |
|--------|------|-------|-------|------|------|-------|-----|
| pAKT  |     |       |       |      |      |       |     |
| Cav-1  | 0.002 | 0.001 | 0.048 | 0.003 | 0.001 | 0.001 | 0.009 |
| eps15 | 0.020 | 0.001 | 0.002 | 0.009 |       |       |     |
| pERK  | 0.001 | 0.048 | 0.002 | 0.003 |       |       |     |
| MDM2  | 0.001 | 0.003 | 0.009 |       |       |       |     |
| TGF-α | 0.001 |       |       | 0.006 |       |       |     |
| EGF   | 0.009 | 0.001 |       |       | 0.006 |       |     |
| p53   | 0.003 | 0.000 | 0.000 | 0.010 |       |       |     |
| c-kit | 0.000 | 0.003 | 0.010 | 0.004 | 0.044 |       |     |
| Cyclin E | 0.019 | 0.036 |       |       |       |       |     |
| Cyclin A |      |       |       |       |       |       |     |
| p21   | 0.006 |       |       |       |       |       |     |
| EGF   | 0.001 |       |       |       |       | 0.011 |     |
| Ki-67 | 0.006 | 0.006 | 0.006 | 0.001 |       |       |     |
| CA-SSR I | 0.022 | 0.050 |       |       |       |       |     |
| tumour grade | 0.002 | 0.000 | 0.000 | 0.000 |       |       |     |

Table 5
Overview about the frequencies of immunohistochemical staining of the investigated molecular markers

| Location of expression | Frequency of marker expression in stromal cells |
|------------------------|-----------------------------------------------|
|                        |                  | Cytoplasm | Nucleus |
| Caveolin               | stroma & epithelium | 17% (9/53) | – |
| Eps15 (H896)           | stroma & epithelium | 19.6% (10/51) | – |
| pAKT                  | stroma & epithelium | 8.6% (5/55) | 10.9% (6/55) |
| pERK                   | stroma & epithelium | 7.1% (4/56) | – |
| EGF                    | mostly epithelium | 18.9% (10/53) | – |
| TGF-α                  | mostly epithelium | 20.4% (11/54) | – |
| mdm2                   | mostly epithelium | – | 20.4% (11/54) |
| tumour grade           |                  | 0.002 | 0.000 | 0.000 |

Caveolin-1 and eps15 on the RNA level was seen predominantly in PT with amplifications in CA-SSR I. This association between egfr intron 1 gene dosage and c-kit expression could also be verified on the protein level. Caveolin-1, as a major structural protein of caveolae, is involved in the storage of EGFR molecules and the mediation of specific EGFR-signals in mesenchymal cells [33]. After EGF stimulation the content of EGFR in caveolae rapidly decreases [20]. This is followed in fibroblasts by an internalization of the receptor, mainly mediated by eps15 [4,29,30]. The transfer of these findings on the functional level therefore points to an increased activity and consequently turnover of EGFR in egfr amplified tumours. It is noteworthy that this especially holds true in PT with a similar EGFR expression. This is further supported by the significant correlation between EGF and TGF-α, as major ligands of EGFR, caveolin-1 as seen before [12], and a differential expression of 10 more genes (Table 3), all described to be involved in EGFR signalling at various stages of internalization and signalling. It is important to note that all previous studies on EGFR expression in PT, but also in other mesenchymal tumours [10], accented predominantly a cytoplasmic EGFR staining pattern.

Within recent years, the importance of caveolin-1 in tumour progression, especially in relation to egfr amplification and overexpression, has become obvious [12]. Caveolin-1 has been described as a major regulator of phosphorylated Akt (pAkt) through inhibition of distinct phosphatases [17]. Our tissue microarray results support these findings. pAkt expression was significantly associated with eps15, caveolin-1, EGF and TGF-α expression in this study. Since this change could not be observed in gene chip analysis it is likely that an increase of pAkt half-time, as previously reported, is responsible for this correlation. However, the
survival advantage mediated by pAkt might be seen on various stages. The pro-proliferate effect might be reflected in the positive correlation with pErk \((p < 0.01)\), but anti-apoptotic effects have also been associated with pAkt \([18]\). Therefore, the interplay between EGFR, caveolin-1 and pAkt represents a reasonable and logical explanation for essential tumour biological features of a subset of PT. However, this model is furthermore able to integrate other previous findings on the overexpression of receptor tyrosine kinases in PT \([7,34]\). For example IGF-R and c-kit have also been shown to be involved in the progression of PT \([26,27]\). Especially for IGF-R, an interplay with caveolin-1 and Akt has been described in detail in other cellular models \([24,25,28]\). Therefore, one might speculate that the caveolin-1/pAkt – axis activated by a number of receptor tyrosine kinases might represent the central key element in the progression of PT. Even though metastases in PT are rare, the inhibition of this putative molecular “bottle neck” might be a therapeutic option for these clinically advanced tumours in the future \([11]\).

Since a functional connection has also been described between pAkt and \(\beta\)-catenin \([22]\), an increase of pAkt might further give an explanation for the association of \(\beta\)-catenin with the progression of PT \([26]\).

In order to find additional support for our hypothesis, we investigated the expression status of mdm2 in PT. This was based on reports that mdm2 expression is mediated by growth factor stimulation and/or genetic alterations such as genetic amplifications \([5,16]\) via Akt \([2]\). Our immunohistochemical results support this interpretation since we could show a significant correlation between nuclear mdm2 and pAkt \((p < 0.01)\). The transfer of mdm2 into the nucleus is mainly due to phosphorylation by pAkt \([19]\). In addition, mdm2 expression was also associated with expression of EGFR, caveolin-1, eps15, p53 and p21. Taking into account the previous observation of a significant correlation between EGFR and p53 expression, the presumed pro-apoptotic effect of p53, due to the genetic instability reflected in egfr amplifications, may be strongly counterbalanced by mdm2 as negative regulator of p53 \([6]\). These results are in contrast with previous reports on mdm2 expression in PT \([14]\). However, from a biological point of view the expression of mdm2 in these tumours could further logically explain the expression of Hif-1{\(\alpha\)} in phyllodes tumours \([1,15]\).

In summary, amplifications of egfr are associated with an increased turnover of EGFR and consequently an increase of pAkt, mdm2 pERK and p53 via caveolin-1 in breast phyllodes tumours. Since this pathway has also been described for other growth factor receptors, it might be that this pathway represents a central, dominating pathway in phyllodes tumours and allows a unifying concept for the pathogenesis of phyllodes tumours.

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