Tyrosine Kinase Inhibition in HPV-related Squamous Cell Carcinoma Reveals Beneficial Expression of cKIT and Src

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Abstract. *Background/Aim: Therapeutic options of locally advanced or metastatic head and neck squamous cell carcinoma (HNSCC) are limited. Src and cKIT are key protein regulators for local tumor progression. The aim of the study was to investigate the therapeutic potential of targeted therapies in human squamous cell carcinoma (HNSCC) in vitro. Therefore, the influence of the selective tyrosine kinase inhibitors niotinib, dasatinib, erlotinib, gefitinib and afatinib on Src and cKIT expression in Human papilloma virus (HPV)-positive and HPV-negative squamous cancer cells (SCC) was analyzed in vitro. Materials and Methods: ELISA was performed to evaluate the expression of Src and cKIT under the influence of nilotinib, dasatinib, erlotinib, gefitinib and afatinib (10 μmol/l) in HPV-negative and HPV-positive SCC (24-96 h of incubation). Results: Gefitinib significantly increased cKIT expression in HPV-positive and HPV-negative cells whereas nilotinib and afatinib decreased cKIT expression in HPV-positive SCC. The influence of tyrosine kinase inhibitors in HPV-negative SCC was marginal. Surprisingly, Src expression was significantly increased by all tested tyrosine kinase inhibitors in HPV-positive SCC. Conclusion: The results revealed beneficial and unexpected information concerning the interaction of selective tyrosine kinase inhibitors and the tumor biology of HNSCC.*

Squamous cell carcinoma of the head and neck (HNSCC) is the most common cancer of the upper aerodigestive tract with an incidence of more than 680,000 new cases every year.

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HNSCC has a mortality rate of approximately 375,000 cases per year (1). Whereas HNSCC is often associated with a continued abuse of alcohol and tobacco, the rising incidence of oropharyngeal HNSCC is HPV-related (2). High-risk type p16 was detected in about 82% of all HPV-positive HNSCCs (3). It is noteworthy that p16-positive HNSCC shows a better overall survival and 5-year disease-free survival which is highly associated with an improved response to chemo- and radiotherapy (4, 5). HPV-related tumors express viral oncogenes E6 and E7 which are provided by circular viral DNA that encodes two capsid proteins L1 and L2 (6, 7). While E7 binds the cullin 2 ubiquitin ligase complex which results in ubiquitination and degradation of the tumor suppressor retinoblastoma protein RB, E6 leads to ubiquitin related proteolysis of p53 and thus causes dysregulation of the cell cycle as well as the loss of p53 conveyed apoptosis (6, 8).

CD117, also known as cKIT, is a receptor tyrosine kinase that was discovered in human acute myeloid leukemia (AML) cells (9, 10). cKIT is activated by the transmembrane protein stem cell factor (SCF) (11, 12). SCF along with cKIT are implicated in various physiologic processes such as embryogenesis and hematopoiesis and play a role in the proliferation and differentiation of stem cells (9, 13, 14). Various mutations in promoter regions of the cKIT gene lead to malignant transformation of cells through dysregulation of cKIT (15, 16). The dimerization of cKIT activates its tyrosine kinase and results in autophosphorylation (17, 18). cKIT leads to the activation of several downstream signaling pathways like phosphatidyl-inositol-3-kinases (PI3K), Janus kinase/Signal transducer and Activator of Transcription (JAK/STAT), mitogen-activated protein kinase (MAPK) pathways and Src kinases and therefore affects cellular motility, survival and proliferation (9, 16). Several mutations of the *cKIT* protooncogene with a consecutive overexpression of cKIT have been detected in gastrointestinal stromal tumors (GIST) (19). Furthermore, melanomas, ovarian dysgerminomas and HNSCC are associated with either raised cKIT expression levels or mutations of its gene (20-22).

The cytoplasmic non-receptor tyrosine kinase (nRTK), also known as Src, is a membrane-associated protein, coded by the proto-oncogene *SRC* (23-25). Src is one of several nRTKs which belong to the Src family kinases (SFK) (25). All SFKs are structurally related, consist of four Src homology (SH) domains and differ in one specific region of the protein (26). Src is normally kept inactive by phosphorylation of tyrosine 530 (24, 27). Src is activated through RTKs such as platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR) and cKIT or protein tyrosine phosphatases (PTPs) as PTP1B (16, 27, 28). SFKs are involved in various signaling cascades like PI3K and MAPK. SFKs can also activate STAT3 which affects cellular survival, differentiation, proliferation and angiogenesis (23). Moreover, the interaction of Src with focal adhesion kinase (FAK), several integrins and the cadherin-catenin complex influences cellular migration, motility, adhesion as well as invasion (23, 24).

The dysregulation of several tyrosine kinases was found to influence the development of several oncological diseases (29). Thus, targeted therapies by selective inhibition of tyrosine kinases have been developed for various types of cancers (30-32). Up to date, targeted therapies have been introduced in the treatment of HNSCC and are currently under investigation in ongoing clinical Phase III trials (33-35). Afatinib selectively and irreversibly binds EGFR as well as other EGFR kinases such as Her2/neu, Her3 and Her4 (36). In contrast to erlotinib and gefitinib, afatinib binds irreversibly to various EGFR family members and is rather able to escape resistance mechanisms (37). As a result, afatinib has been shown to provide significant clinical benefits in the therapy of non-small cell lung cancer (NSCLC) and is approved in patients with mutated EGFR (36, 38, 39). Dasatinib targets BCR/ABL as well as cKIT and Src and can be used as a second-line therapy of chronic myeloid leukemia (CML) (40). Erlotinib as well as gefitinib are reversible inhibitors of the tyrosine-kinase domain of EGFR and are used in patients with activating *EGFR* mutations in NSCLC (41, 42). Erlotinib can be also used in patients with metastasized pancreatic carcinoma. Nilotinib targets BCR/ABL, cKIT and PDGFR and can be applied to patients with CML with the philadelphia chromosome or when refractory to conventional treatment options (43, 44). This raises the question whether these five tyrosine kinase inhibitors could also be suitable for the therapy of HNSCC. The aim of this study was to investigate the expression of cKIT and Src in HPV negative and positive squamous cell carcinoma (SCC) under the influence of the tyrosine kinase inhibitors nilotinib, dasatinib, erlotinib, gefitinib and afatinib *in vitro*.

Materials and Methods

Cell lines, drugs and study design. The HPV negative HNSCC cell lines were kindly provided by T.E. Carey, Ph.D. University of Michigan, Ann Arbor, MI, USA. HNSCC 11A cell line originated from a primary squamous cell carcinoma of the epiglottis, whereas HNSCC 14C originated from a skin metastasis of an oral SCC after radiation, chemotherapy and surgery. The CERV196 cell line is positive for HPV 16 and was provided from poorly differentiated SCC of the uterine cervix and acquired from Cell Lines Service GmbH, Eppelheim, Germany. HPV negative cells were cultured with Eagle's minimum essential medium (Gibco, Life Technologies, Carlsbad, CA, USA) and supplemented with 2mM of L-glutamine, 10% fetal calf serum and Pen-Strep (Gibco, Life Technologies). Cultured HPV positive cells were supplemented with 2mM L-glutamine, 1.0 g/l sodium bicarbonate, 1.0 g/l sodium pyruvate, 0.1 mM non-essential amino-acids and 10% of fetal bovine serum (Gibco, Life Technologies). Cell cultures were grown under standardized conditions (37°C, 5% CO_2, 95% humidity). For subcultures 0.05% trypsin/0.02% EDTA solution was added for 5 minutes at 37°C (Sigma Aldrich, St. Louis, MO, USA). Incubation time ranged from 24 to 96 h. Nilotinib, dasatinib, gefitinib, erlotinib and afatinib were provided by the Oncological Department, University Hospital Mannheim GmbH. Substances were dissolved in dimethylsulfoxide at a concentration of 10 μmol/l. Cell proliferation assay was performed in 96-well microtiter plates (alamarBlue©, AbD Serotec, Oxford, UK).

Enzyme-linked immunosorbent assay (ELISA) for cKIT and Src. To determine the protein concentrations of cKIT and Src sandwich ELISA technique was applied. For both proteins, DuoSet ELISA development kits (R&D Systems, Inc., Minneapolis, MN, USA and Bio-Techne GmbH, Wiesbaden, Germany) were used (DY332 for cKIT and DYC2685 for Src) and performed in accordance to the manufacturer's instructions. The optical density was measured at a wavelength of 450 nm with wavelength correction set to 540 nm with a MRX Microplate Reader (DYNEX Technologies, Chantilly, VA, USA). Concentrations were determined in pg/ml and the detection range was 31.2-2000 pg/ml for cKIT and 3.91-250 pg/ml for Src. The inter-assay coefficient of variation reported by the manufacturer was <10%.

Statistical analysis. The statistical analysis was performed using the mean values for each experiment. Each experiment was independently performed for three (n=3) times. The means were compared to the mean values of the negative control using the two-coefficient variance test to assess statistical significance (SAS Statistics software, version 9.3; SAS Institute, Inc., Cary, NC, USA). The resulting *p*-values were adjusted by using Dunnett's test. For all analyses, a *p*-value ≤0.05 was defined as statistically significant. The statistical analysis was performed in collaboration with Prof. Dr. C. Weiss, Institute of Biomathematics, Medical Faculty Mannheim, University of Heidelberg, Germany.

Results

cKIT expression in HNSCC 11A, 14C and CERV196. cKIT expression was observed in all three cell lines tested, reaching the highest expression levels in HPV positive CERV196 after 96 h. All tested substances led to an alteration of cKIT expression. In CERV196, the expression of cKIT increased in a time-dependent manner in both, untreated cultures and after treatment with tyrosine kinase inhibitors. One exception can be stated after treatment with gefitinib as cKIT expression after 48 h was slightly reduced compared to 24 h. However, after 48 h of incubation, cKIT expression was significantly

Table I. *cKIT expression in HPV-negative HNSCC 11A and 14C and HPV-positive CERV196 after incubation with gefitinib, erlotinib, dasatinib, nilotinib, and afatinib (10 μmol/l). Data are mean values [pg/ml].*

Incubation time (h)	Negative control	Gefitinib (10 μmol/l)		Erlotinib (10 μmol/l)		Dasatinib (10 μmol/l)		Nilotinib (10 μmol/l)		Afatinib (10 μmol/l)	
	Mean	Mean	*p*-Value	Mean	*p*-Value	Mean	*p*-Value	Mean	*p*-Value	Mean	*p*-Value
HNSCC 11A											
24 h	15.53	18.47	0.466	17.77	**0.026**	18.17	0.093	16.27	0.837	15.37	0.969
48 h	14.60	19.30	**0.015**	13.07	0.683	17.20	0.440	15.47	0.849	19.17	**0.020**
72 h	18.90	24.00	0.664	15.50	**0.029**	18.57	0.982	20.87	**0.048**	14.33	**0.005**
96 h	15.40	19.60	0.003	19.50	**0.006**	15.73	0.962	14.60	0.453	17.37	0.397
HNSCC 14C											
24 h	14.30	18.07	0.052	14.10	0.994	12.73	0.188	13.70	0.812	11.87	0.154
48 h	16.33	16.20	0.999	17.07	0.816	17.10	0.934	16.13	0.992	13.63	0.299
72 h	15.23	18.30	**0.008**	14.67	0.731	14.53	0.701	13.80	0.300	14.50	0.910
96h	15.50	14.60	0.875	14.30	0.758	17.00	0.487	16.23	0.928	14.33	0.765
CERV196											
24 h	12.13	19.43	0.008	16.70	0.077	17.63	**0.003**	13.60	0.387	13.77	0.700
48 h	17.30	19.33	0.632	18.53	0.689	18.37	0.370	14.30	0.385	19.00	0.086
72 h	33.33	41.97	**0.008**	29.97	0.194	34.47	0.940	25.63	**<0.001**	21.97	**<0.001**
96 h	43.53	66.53	**<0.001**	34.60	**0.018**	42.57	0.580	29.57	**<0.001**	28.43	**<0.001**

Statistically significant differences (p≤0.05) in bold.

elevated compared to the negative control (*p*=0.008). Gefitinib led to an increased cKIT expression in all three cell lines with a statistically significant increase after 48 (*p*=0.015) and 96 (*p*=0.003) h in HNSCC 11A, after 72 (*p*=0.008) h in HNSCC 14C and after 24 (*p*=0.008), 72 (*p*=0.008) and 96 h (*p*<0.001) in HPV-positive CERV196. Erlotinib significantly altered cKIT expression only in HNSCC 11A and CERV196. In CERV196 the expression was significantly decreased after 96 (*p*=0.018) h, whereas a statistically significant decrease of cKIT levels in HNSCC 11A were only observed after 72 (*p*=0.029) h. Surprisingly, erlotinib led to a statistically significant increase in cKIT after 24 (*p*=0.026) and 96 (*p*=0.006) h. The expression pattern of cKIT in erlotinib treated cells in HNSCC 14C was similar to those of the negative control of HNSCC 14C. The influence of dasatinib on cKIT expression in all tested cell lines was negligible with one exception in CERV196 after 24 h when cKIT expression was significantly elevated compared to the negative control (*p*=0.003). Nilotinib revealed interesting results regarding cKIT expression. Whereas nilotinib significantly increased cKIT expression in HPV negative HNSCC 11A after 72 (*p*=0.048) h, a significant decrease in cKIT expression in HPV positive CERV196 was observed after 72 (*p*<0.001) and 96 (*p*<0.001) h. A statistically significant decrease in cKIT expression was also observed after treatment with afatinib in CERV196 after 72 (*p*<0.001) and 96 (*p*<0.001) h, whereas the expression of cKIT was decreased after 72 (*p*=0.005) in HNSCC 11A, however increased after 48 (*p*=0.02) h. Interestingly, in HNSCC 14C, a significant effect on cKIT

expression was only seen after gefitinib treatment. It is noteworthy that gefitinib was the only substance that increased the expression of cKIT independent of the HPV status. Data are displayed in Table I and Figure 1.

Src expression in HNSCC 11A, 14C and CERV196. SRC expression was observed in all three cell lines tested. In the negative control of HPV negative HSNCC 11A cells the expression of Src increased time-dependently whereas fluctuating levels of Src were detected in the other tested cell lines. With one exception after treatment with gefitinib, the expression of Src increased statistically significantly after exposition to all tested substances in HNSCC 11A (*p*≤0.036). This effect was observed after 24 h of treatment (*p*≤0.007). Gefitinib had no significant effect on Src expression in the HPV negative HNSCC 11A. However, in HPV negative HNSCC 14C gefitinib led to a significant reduction in Src levels after 96 h (*p*=0.009). In HPV-positive CERV196, gefitinib significantly increased Src expression after 24 (*p*<0.001) and 96 h (*p*=0.005). It is noteworthy, that this gefitinib-induced increase after 24 h marks the highest level of Src expression in CERV196. Erlotinib increased Src levels only after 24 h in HNSCC 11A (*p*=0.003) and in HPV positive CERV196 (*p*=0.008). In HPV negative HNSCC 14C, erlotinib had no significant impact on Src expression. In HPV negative HNSCC 11A, dasatinib led to a statistically significant increase after 24 and 48 h (*p*=0.002 and *p*=0.036). It is noteworthy that dasatinib was the only tested substance that caused a decrease in Src expression in
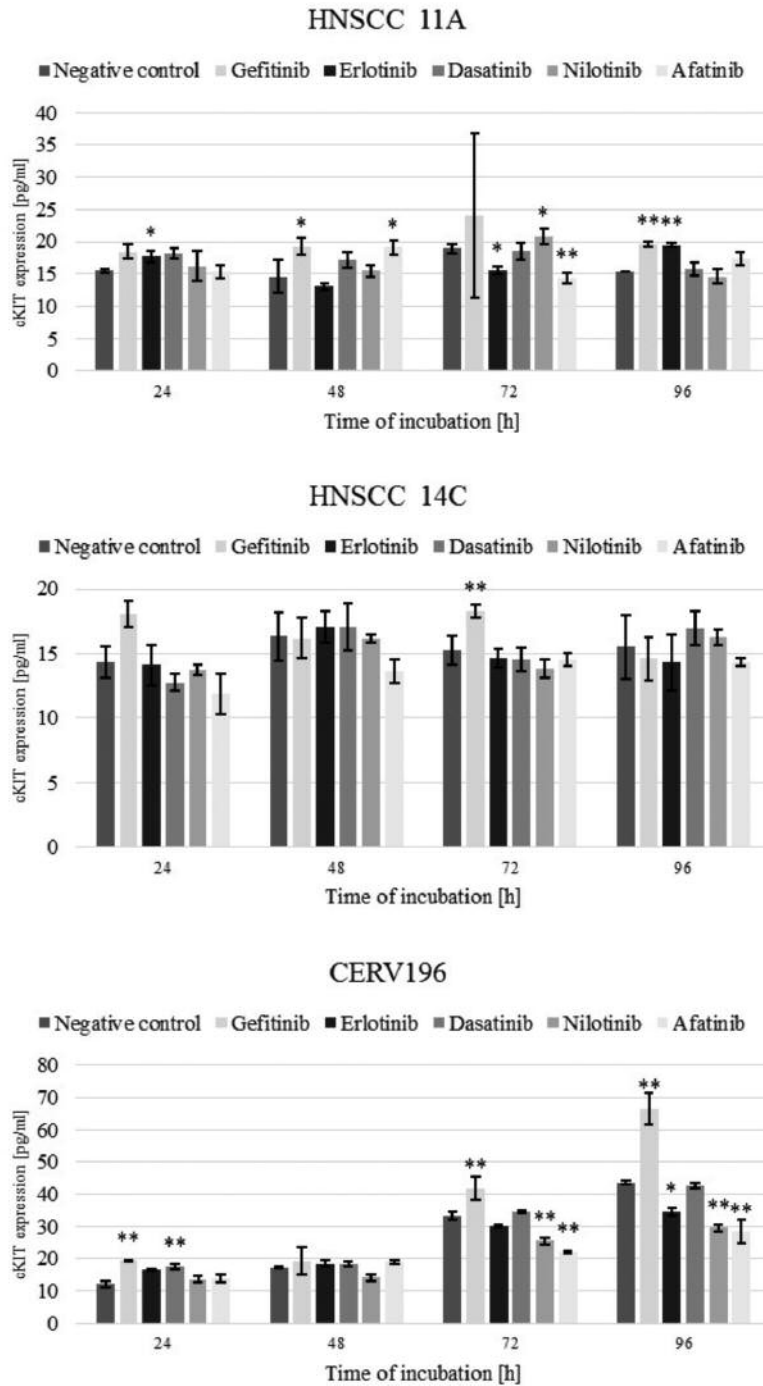
HNSCC 11A

HNSCC 14C

CERV196

Figure 1. *cKIT expression in HNSCC 11A, 14C and CERV196 after incubation with gefitinib, erlotinib, dasatinib, nilotinib, and afatinib at 10 μmol/l compared to the negative control. Data are mean values. Standard deviation is indicated (*p<0.05, **p<0.01).*

HNSCC 11A after 96 h ($p=0.002$). Dasatinib had no significant impact on Src expression in HNSCC 14C but caused a significant increase in Src after 24 h in HPV positive CERV196 ($p=0.043$). Nilotinib led to a significant increase in Src only after 24 h in HNSCC 11A ($p=0.002$) and

after 96 h in the CERV196 ($p=0.01$). Nilotinib was the only tested drug that showed no effect in the CERV196 after 24 h. Src expression in HNSCC 14C was not affected by nilotinib compared to erlotinib and dasatinib. Afatinib influenced Src expression in all tested cell lines. It caused a

Table II. *Src expression in HPV-negative HNSCC 11A and 14C and HPV-positive CERV196 after incubation with gefitinib, erlotinib, dasatinib, nilotinib, and afatinib (10 μmol/l). Data are mean values [pg/ml].*

Incubation time (h)	Negative control	Gefitinib (10 μmol/l)		Erlotinib (10 μmol/l)		Dasatinib (10 μmol/l)		Nilotinib (10 μmol/l)		Afatinib (10 μmol/l)	
	Mean	Mean	*p*-Value	Mean	*p*-Value	Mean	*p*-Value	Mean	*p*-Value	Mean	*p*-Value
HNSCC 11A											
24 h	11.40	27.13	0.063	37.60	**0.003**	29.07	**0.002**	27.23	**0.002**	26.30	**0.007**
48 h	13.30	12.07	0.961	19.93	0.613	26.43	**0.036**	15.73	0.813	29.53	**<0.001**
72 h	27.43	22.83	0.733	15.87	0.175	25.10	0.797	30.73	0.142	19.17	0.088
96 h	34.47	30.97	0.553	30.73	0.754	9.07	**0.002**	12.07	0.114	38.67	0.811
HNSCC 14C											
24 h	27.60	18.93	0.766	28.30	1.000	28.47	0.999	29.87	0.990	20.87	0.695
48 h	25.43	25.40	1.000	30.27	0.551	32.10	0.255	25.53	1.000	23.47	0.926
72 h	24.60	26.53	0.910	26.83	0.940	30.77	0.142	22.00	0.527	27.83	0.367
96 h	34.90	17.07	**0.009**	24.13	0.277	32.70	0.901	34.80	1.000	24.70	**0.030**
CERV196											
24 h	16.10	36.60	**<0.001**	29.57	**0.008**	23.10	**0.043**	25.33	0.367	29.00	**0.033**
48 h	20.63	19.43	0.992	26.10	0.526	11.47	0.174	17.40	0.820	16.00	0.313
72 h	28.27	21.93	0.498	29.50	0.994	32.27	0.457	32.60	0.465	21.87	0.348
96 h	19.07	28.57	**0.005**	20.00	0.994	18.00	0.992	31.03	**0.010**	30.93	**0.005**

Statistically significant differences (p≤0.05) in bold.

significant increase in Src expression in HPV negative HNSCC 11A and HPV positive CERV196; in HNSCC 11A after 24 and 48 h ($p=0.007$ and $p<0.001$) and in CERV196 after 24 and 96 h ($p=0.033$ and $p=0.005$). In HNSCC 14C afatinib led to a significant reduction in Src after 96 h ($p=0.03$). Data are displayed in Table II and Figure 2.

Discussion

This study was designed to investigate the expression of cKIT and Src in HPV positive and HPV negative SCC after treatment with the tyrosine kinase inhibitors gefitinib, erlotinib, dasatinib, nilotinib and afatinib. cKIT and Src play a role in local tumor progression and are key regulators of cell motility and survival (9, 23). Targeted therapy is established in several cancer entities and the evaluation on SCC protein expression patterns may give essential insights into the cellular signaling in HNSCC as well as possible new strategies to alternative therapeutic approaches (30-32).

cKIT expression was observed in all tested cell lines and the highest expression levels were found in HPV positive CERV196. This observation is interesting as it suggests an even stronger elevation of cKIT expression in HPV positive SCC compared to increased cKIT expression in HPV negative HNSCC (20). Furthermore, it indicates an increased activity of cKIT related signaling pathways in HPV positive SCC. Thus, our results are in accordance with the findings of de Melo Maia *et al*. who stated that vulvar tumors with high cKIT expression levels are associated with a better prognosis

(45). One possible explanation may therefore include an elevated cKIT expression in HPV positive tumors. For all tested cell lines, a statistically significant effect of TKI treatment was detected regarding cKIT expression. However, in HPV negative HNSCC 14C an alteration in cKIT expression was only observed after treatment with gefitinib for 72 h. In contrast to this observation, cKIT expression in HNSCC 11A has been variously alternated by all tested TKI despite dasatinib. A possible explanation is that HNSCC 14C is originated from metastatic tissue and might be less susceptible to tyrosine kinase inhibition than HNSCC 11A cells which are derived from a primary epiglottis SCC due to mutations in the target protein. This might also explain why both TKI dasatinib and nilotinib, which act as direct cKIT inhibitors, have limited effects in HNSCC 14C regarding cKIT expression (40, 43). However, cKIT expression in HNSCC 11A was not significantly affected by dasatinib as in HPV positive CERV196. This indicates that HPV related SCC could be rather sensitive to TKI inhibition compared to HPV negative SCC. Another explanation for this observation is that CERV196 was the only tested cell line which led to a significant alteration in cKIT after treatment with all applied TKI. This is relevant as cKIT inhibition facilitates a decreased tumor growth as well as increased cell survival in several tumor entities (19, 46).

The non-receptor tyrosine kinase Src is normally kept inactive but can be activated through RTKs like EGFR or PDGFR. Once activated, Src can interact with various RTKs, FAKs and other cellular regulators (47). Src is,
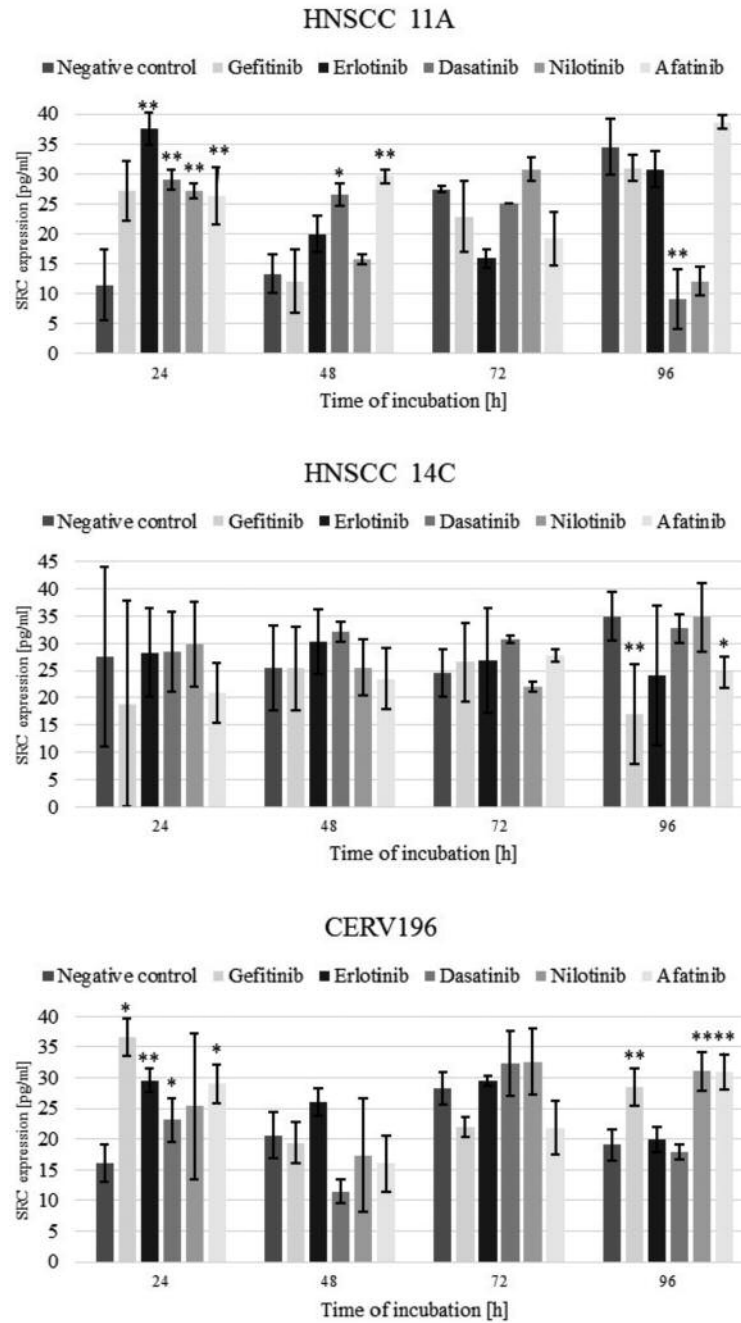
HNSCC 11A

HNSCC 14C

CERV196

Figure 2. *Src expression in HNSCC 11A, 14C and CERV196 after incubation with gefitinib, erlotinib, dasatinib, nilotinib, and afatinib at 10 μmol/l compared to the negative control. Data are mean values. Standard deviation is indicated (*p<0.05, **p<0.01).*

therefore, involved in cellular migration, motility, adhesion and invasion (23, 24). As already published in previous works, protein expression can be substantially influenced by selective TKIs in HPV related SCC (48-51). Src expression could be observed in all tested cell lines but not all tested TKIs showed an effect on Src expression. In HPV negative HNSCC 11A, all tested substances except of gefitinib led to

a significant alteration in Src expression. Yet, only erlotinib which is structurally related to gefitinib significantly increased Src expression after 24 h (41, 42). These findings are surprising as Src acts downstream of EGFR and can be activated through this receptor tyrosine kinase (52). This is possible due to the fact that Src is not affected by selective EGFR inhibition in HPV negative HNSCC. Distinct effects

on Src expression were not observed after treatment with erlotinib as well as gefitinib in HNSCC 14C and HPV positive CERV196. Only gefitinib significantly decreased Src expression in HNSCC 14C after 96 h. This leads to the question whether selective EGFR inhibiting proteins can induce drug resistance in HNSCC through potential predictive biomarkers. Stabile *et al*. demonstrated that Src activation is an indicator of erlotinib resistance in HNSCC (53). These observations can be interpreted as another indication for the assumption why HNSCC 14C cells are less responsive to tyrosine kinase inhibition. Concerning dasatinib as a direct Src inhibitor, the expression of Src in HNSCC 11A increased after 24 and 48 h but then decreased significantly after 96 h (40). This is notable, since no other combination of cell line and treatment has led to both, an increase and a decrease in Src expression levels. Dasatinib may act as a direct inhibitor of Src particularly in HPV negative HNSCC 11A cells, as they originate from primary epiglottis SCC. Yet, it remains unclear how HNSCC 14C and HPV positive CERV196 cancer cells react to dasatinib treatment. It has been demonstrated that acquired EGFR resistance is responsible for the refractory behavior of squamous cancer cells to EGFR inhibiting substances (54). This may be why Src as a downstream effector of EGFR, could be difficult to target. However, it has been shown that Src family kinases are activated in cetuximab-resistant cells that could be resensitized to cetuximab by using dasatinib (55). Another option would therefore be a combination of Src inhibition and selective EGFR inhibition (56). Moreover, the activation of EGFR might be a regulator of dasatinib mediated effects. Increasing levels of activated EGFR could prevent dasatinib-mediated apoptosis through Src (57). Regarding HPV positive CERV196, all tested TKIs led to a significant alteration in Src expression. As previously suggested, HPV-positive tumors might be more responsive to selective tyrosine kinase inhibitors compared to HPV-negative SCC. However, it is remarkable that the expression of Src was significantly increased by the TKIs. A possible explanation for the increased Src expression in HPV-positive cancer cells could be the yet undetected evasive mechanisms of these cells through oncogenic proteins such as E6 and E7 or through HPV-related autocrine release of cell stabilizing proteins that prevent apoptosis (58). Finally, yet unknown mechanisms of drug resistance could affect chemosensitivity in HPV related squamous cancer (59).

To our knowledge, this is the first study investigating the influence of gefitinib, erlotinib, dasatinib, nilotinib and afatinib on the expression patterns of cKIT and Src in HPV positive and HPV negative SCC *in vitro*. Significant changes in cKIT and Src expression after treatment with these tyrosine kinase inhibitors in both, HPV negative and HPV positive SCC were detected. In addition, the results provide

novel aspects in the interaction of cKIT and Src with small molecule tyrosine kinase inhibitors in HNSCC *in vitro*. The results also reveal possible innovative approaches for further investigations of potential new strategies in targeted therapy of HNSCC.

Acknowledgements

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