Predictors of sensitivity and resistance mechanisms

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Session: Biologically based Treatment in head and neck squamous cell carcinoma
Disclosure slide

• Advisory Board (Merck KGaA)
Potential mechanisms of resistance to EGFR-targeted therapies in HNSCC

Mechanisms of resistance

- EGFR mutations
- Ras mutations
- Epithelial-mesenchymal transition

Examples

- Extracellular domain (EGFRvIII)
- H-ras mutations
- Aberrant cortactin expression, delta-crystallin enhancer binding factor 1 (E-cadherin repressor)
- Cyclin D1 upregulation
- PTEN mutations or decreased expression
- PI3KCA mutations, Akt amplification, EGFR phosphatase (PTPRS)
- Induction of alternative oncogenic pathways by EGFR blockade (i.e. STAT3)
- G-protein-coupled receptor mediated activation of EGFR (i.e. PDK1)
- Concomitant activation of Met, Her2, IGF-1R, Src kinases
Predictive biomarkers for response to EGFR-targeted therapies
High tumor EGFR protein by IHC tended to be associated with reduced PFS in the cetuximab-treated cohort.

ERK

• Predictors for response to cetuximab in a prospective clinical trial (E2303) of patients (pts) with operable stage III/IV HNSCC phase II trial were analyzed on a tissue microarray

• EGFR, ERK1/2, Met, pAkt and STAT protein expression levels were assessed using automated quantitative protein analysis (AQUA)
Progression-Free Survival and Overall Survival by ERK Status

Psyrri et al: ASCO 2012
Mutational analysis of HRAS

Mutation detection analysis showed that 11 of 158 (6.96%) HNSCC specimens contained mutations at hotspot codons 12,13 and 61.
We also detected two mutations in codon 14 (V14M), one in codon 17 (S17N), one in codon 54 (D54G) and one in codon 63 (E63K). Our analysis showed that 3.16% HNSCC samples contained rare HRAS mutations.

In total, HRAS mutation analysis showed that 10.13% HNSCC specimens harbor mutations in HRAS gene that affect the protein function and specificity.
HRAS status and clinical outcome

- Patients bearing tumors with mutated HRAS had inferior mean OS (22.13 vs 35.20, \( p=0.02 \)) and a non-significant trend for inferior mean DFS.

- Patients had received various treatments such as surgery plus/minus RT and various chemotherapy regimens. A subgroup analysis of 38 patients treated with cetuximab-based regimens showed that wt Hras was associated with higher likelihood of attaining CR or PR to treatment of borderline significance (\( p=0.06 \)) due to small sample size.
EGFR 7p12
80-90% overexpression
20-25% overactivity

EGFR phosphorylation

PI3K 74%

PI3KCA 3q26
40% amplification
10% mutation

PTEN 10q23
5-10% genetic alterations
30% decrease expression

HRAS 7% mutation

Survival
Proliferation
Oncogenesis

Survival
Proliferation
Oncogenesis

Cell cycle progression

Transformation
Differentiation
Apoptosis

BCL-X, MYC

Cyclin D1

Cell membrane

Cytoplasm

Nucleus
RAS and PI3K

• We have developed a model cell system to study the impact of HRAS and PIK3CA mutations in cetuximab resistance in HNSCC.

• To investigate whether activating mutations in downstream targets of EGFR can lead to resistance to EGFR blockade in head and neck cancer, we infected a group of cetuximab resistant HNSCC cell lines bearing mutations in HRAS (BB49) or PIK3CA (Cal-33) and a group of wild-type HRAS/PI3K cetuximab resistant HNSCC cell lines (UM-SCC-11A, UM-SCC-6) with lentivirus expressing shRNA that targets the HRAS gene or control shRNA.
HRAS silencing did not affect the expression levels of EGFR, p-EGFR, AKT and ERK1/2. However, HRAS downregulation was found to be associated with a significant reduction of phospho ERK1/2 and phospho AKT in HRAS/PI3K mutated cell lines.
HRAS silencing in combination with Cetuximab treatment

**MTT VIABILITY ASSAY**

Treatment with 50 nM Cetuximab suppressed almost completely the proliferation of HRAS/PI3K mutated compared to HRAS/PI3K wild type HNSCC cell lines that were infected by LV_##65shRNA.
Cetuximab plus HRAS silencing

Un: Uninfected
LV_Scr: Infected with lentivirus expressing control shRNA
LV_shRNA#65: Infected with lentivirus expressing shRNA #65

Cetuximab: (50nM)

BB49

CAL-33

p-AKT (Ser 473)
AKT
p-STAT3 (Tyr705)
STAT3
b-actin
A direct comparison of the proliferative rate between cetuximab treated and untreated LV_#65shRNA infected BB49 and Cal-33 cells confirmed the restoration of sensitivity to cetuximab in these cells after HRAS silencing.
Molecular crosstalk between EGFR, RAS, PI3K pathways
Conclusions

• Currently, no biomarker is predictive for response to cetuximab in HNSCC

• *HRAS* genetic alteration is a frequent event in HNSCC

• Cell lines bearing *HRAS* mutation are resistant to cetuximab and *HRAS* silencing renders these cells sensitive

• HRAS silencing suppresses the ability of activated PI3K to promote the phosphorylation of AKT
Thank you