EGFR EXPRESSION AND AUTOPHAGY MODULATION IN GLIOBLASTOMA: IN VITRO RESULTS AND RELEVANCE FOR A CLINICAL SERIES

Paolo Tini 1,3, Silvia Palumbo1,3, Marzia Toscano1,3, Pierpaolo Pastina1,3, Clelia Miracco1,4, Luigi Pirtoli1,3

1 Istituto Toscano Tumori, Florence, Italy
2 Department of Biotechnology, University of Pavia, Italy
3 Department of Radiation Oncology, University Hospital of Siena, Italy.
4 Department of Human Pathology, University Hospital of Siena, Italy.
Locus amplification, gene overexpression, and genetic mutations of epidermal growth factor receptor (EGFR) exist in the majority of Glioblastomas (GB).

Consequent modifications of its downstream signal pathway, involve also the down-regulation of the autophagy process, thus promoting the aggressive phenotype and radiation resistance of this tumor.
We analyze the interactions between EGFR expression and autophagy in GB throughout IN VITRO experiments and its relevance in the CLINICAL setting.
**Method and Materials**

- **IN VITRO MODULATION of EGFR and AUTOPHAGY:**

  - EGFR expression profile and autophagy were modulated in human T98G and U373MG-GB cell cultures.
  
  - Cell migration capability was evaluated by a migration assay.
  
  - Radiosensitivity was tested by clonogenic assay after IR treatment.
  
  - Inhibition of EGFR expression and autophagy was achieved by specific siRNAs (siEGFR and siATG7).
  
  - Autophagy induction was obtained with a m-TOR inhibitor (Rapamycin.)

Method and Materials

**RELEVANCE IN THE CLINICAL SETTING EGFR and AUTOPHAGY MARKERS:**

- The clinical relevance of these markers was investigated out of a series of 156 consecutive GB patients undergoing a standard RT-TMZ protocol.

- We analyzed the expression of EGFR (156 pts) and of the autophagy protein Beclin-1 (Beclin-1 Protein Cytoplasmatic Expression: BPCE) (81pts) on surgical samples.

- We clustered patients according an expression score for EGFR and BPCE.

- EGFR expression and BPCE were analyzed to find out potential correlation with incidence of multifocal disease and Overall survival (OS).
Method and Materials

**EGFR expression score**

✓ A Neuropathologist evaluated EGFR expression on surgical samples by IHC:

\[ IHC \text{ Intensity} + \% \text{ positive cells} = \text{EGFR expression score} \]

- Low-negative EGFR expression
- High EGFR expression
Method and Materials

**Beclin-1 protein cytoplasmic expression score (BPCE)**

- A Neuropathologist evaluated the autophagy protein Beclin-1 expression score on surgical samples by IHC:

  - **IHC Cytoplasmatic Intensity**
  - **BPCE score**
  - **Low BPCE**
  - **High BPCE**
Results

EGFR silencing by a four siRNA pool, mTOR inhibition by Rapamicyn, and IR (2 Gy). Clonogenic tests in T98G and U373MG cell lines.
Results

• In vitro, combined si-EGFR and Rapamicin induced a decrease in cellular migration rates.

• In vitro, combined si-EGFR and Rapamicin increased IR response.
Results

Clinical and pathological characteristics

*Patients: n°156 (EGFR and multicentric/unicentric disease)*

- In high EGFR expression group: we found an increased incidence of multicentric disease ($p=0.03$)
Results

✓ MRI-based tumor growth evaluation according to EGFR expression subgroups (Low EGFR vs. High EGFR expression)
**Results**

**Clinical and pathological characteristics**

*Patients: n°81 (CLUSTERED FOR EGFR EXPRESSION and BPCE)*

*Follow-up (mean): 12 months*

- **In high EGFR group, BPCE didn’t correlated with OS (p=0.163).**
- **In low-negative EGFR group, BPCE strongly correlated with OS: median 18 months in low-BPCE versus 35 months of in high-BPCE pts (p=0.02)**
Conclusion

✓ **Our results suggest that the relationship between EGFR expression and autophagy regulation could play a key role in invasion and growth patterns, and intrinsic radioresistance of GB.**

✓ **A novel combined EGFR-autophagy modulation strategy can be hypothesised, to overcome the intrinsic GB radioresistance, thus improving the effectiveness of current standard of treatments of GB.**

✓ **Other predictive biomolecular markers of local aggressiveness deserve further investigation in GB, for more through mechanistic knowledge of the EGFR-PIK3-AKT-mTOR signal pathway, to this purpose.**
“L’ironia e l’intelligenza sono sorelle di sangue.”