Structural Characterization of Lung Cancer Xenografts for the Assessment of the Therapeutic Efficacy of EGFR Targeting Molecules

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Background. Non-small cell lung cancer (NSCLC), which currently accounts for nearly 85% of primary malignant lung tumors, remains the leading global cause of cancer-related death worldwide. Recently, the goal of oncologic research to improve outcome and reduce treatment-related side-effects has led to the development of novel anticancer treatments targeting specific proteins or genes involved in cancer growth and progression.
The epidermal growth factor receptor (EGFR) is an established target for anti-cancer treatment in different tumors. Two strategies have been explored to inhibit this pivotal molecule in epithelial cancer: small molecules tyrosine-kinase inhibitors, Gefitinib and Erlotinib, and ErbB/HER-targeting monoclonal antibodies such as Cetuximab and Trastuzumab. Although imaging techniques have improved the documentation of tumor regression and/or resistance, severe limitations persist on clinical ground on the real assessment of the therapeutic response. It should be established whether changes in tumor dimensions are the result of an effective reduction of cancer cells or a stromal reaction to the ongoing cell death, or both. This aspect is particularly relevant when antibodies-based target therapy is employed because of the involvement of an immune cell-mediated cytotoxic effect.
Objective. We advanced the hypothesis that the efficacy of different drugs targeting molecular pathways associated with NSCLC implies a differential impact on the cross talk between neoplastic cells and stromal compartments.
Methods. To this purpose a xenograft model of lung adenocarcinoma was produced on 30 female BALB/c-Nude mice subcutaneously injected with 10^7 Calu-3 human cells. When tumors were well established, mice were randomized to receive vehicle (V), Gefitinib 25 (GF25) or 100 (GF100) mg/Kg or Erlotinib (Erl) and Cetuximab (Cet) alone or in combination (Erl+Cet).
A morphometric analysis was performed to evaluate neoplastic cell number, proliferation, necrosis and apoptosis. In addition, the amount of fibrosis and stromal and inflammatory interstitial cells was measured. These latter cell populations were identified, respectively, by anti-vimentin and mouse specific anti-CD45 antibodies. The assessment of the origin of neoplastic and stromal cell compartments within the nodules was performed by FISH analysis of human sex chromosomes (hChr).
Results. Histologic examination showed that the xenograft strikingly reproduces features of human lung adenocarcinoma. Immunohistochemical and FISH analysis documented that neoplastic epithelial cells expressing cytokeratin 7 and CD44 carrying hChr were organized in secretory glands surrounded by cellularized collagen as confirmed by Masson's trichrome staining.
Experimentally, GF100 and Erl+Cet groups resulted in the most significant reduction of tumor size. Regressive phenomena and changes in size of neoplastic glands together with intense stromal reaction were observed in histologic samples of tumors from treated mice.
By morphometric approach, in agreement with gross anatomic measurements, we documented that GF100 determined a reduction of both stromal (6.37±1.95%) and neoplastic (3.08±1.82%, $p<0.05$) compartments compared to V samples (8.17±2.36% and 19.64±10.01, respectively). Conversely, Erl+Cet treatment inhibited tumor growth by reducing neoplastic structures (-40.15% vs V, $p<0.01$) without effect on stromal components. Interestingly, Cet given alone resulted in a more intense inflammatory reaction compared to Erl treated tumors. Moreover, although interstitial cells largely belonged to the recipient mouse, we identified cells carrying hChr occasionally organized in vessel-like structures surrounding neoplastic glands, documenting the involvement of injected cells in stromal tissue composition.
In conclusion, our experimental approach allows to assess the therapeutic efficacy and the differential stromal involvement in tumor regression by several targeted drugs.

References

1. Galvani E, Alfieri R, Giovannetti E, Cavazzoni A, La Monica S, Galetti M, Fumarola C, Bonelli M, Mor M, Tiseo M, Peters GJ, Petronini PG, Ardizzoni A. Epidermal growth factor receptor tyrosine kinase inhibitors: current status and future perspectives in the development of novel irreversible inhibitors for the treatment of mutant non-small cell lung cancer. Curr Pharm Des. 2013;19:818-32

2. Cavazzoni A, Alfieri R, Cretella D, Saccani F, Ampollini L, Galetti M, Quaini F, Graiani G, Madeddu D, Mozzoni P, Galvani E, La Monica S, Bonelli M, Fumarola C, Mutti A, Carbognani P, Tiseo M, Barocelli E, Petronini PG, Ardizzoni A. Combined use of anti-ErbB monoclonal antibodies and erlotinib enhances antibodydependent cellular cytotoxicity of wild-type erlotinib-sensitive NSCLC cell lines. Mol Cancer; 11:91, 2012