**Elucidation of the mechanism of gefitinib-induced immune related adverse reaction and search for biomarkers to predict its pathogenesis**

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Background

The epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs) have been approved for non-small cell lung cancer. Although EGFR TKIs are less toxic than traditional cytotoxic therapies, they cause many severe idiosyncratic drug reactions. Reactive metabolites can cause cellular damage with the release of damage-associated molecular patterns (DAMPs), which is thought to be involved in immune activation. Inflammasomes can be activated by DAMPs, and this may be a common mechanism by which DAMPs initiate an immune response.

Methods

Human hepatocarcinoma functional liver cell-4 (FLC-4) cells were cultured with afatinib, dacomitinib, erlotinib, gefitinib, and osimertinib for 7 days, and then the supernatant was added to differentiated THP-1 cells and incubated for 24 hr. The control was incubation without drugs. IL-1β concentration in the THP-1 culture medium was measured using an ELISA kit. Caspase-1 activity was also measured using the Caspase-Glo® 1 Inflammasome Assay. DAMPs were evaluated by western blotting using the hepatocyte supernatant.

Results

We found that the supernatant from the incubation of gefitinib with FLC-4 cells for 7 days led to increased caspase-1 activity and production of IL-1ß by THP-1 cells. In the supernatant of FLC-4 cells with gefitinib, the heat shock protein (HSP) 40, 70 and 90 were significantly increased. In addition, activated THP-1 cells secreted high mobility group box 1 (HMGB1) protein.

Conclusions

These results support the hypothesis that the reactive iminoquinone metabolite can cause the release of DAMPs from hepatocytes, which in turn, can activate inflammasomes. Inflammasome activation may be an important step in the activation of the immune system by gefitinib, which in some patients, can cause

immune-related adverse events. Detected HSPs may be predictive biomarkers of gefitinib-induced adverse events.

Key Words
Epidermal growth factor receptor tyrosine kinase inhibitors, Gefitinib, Reactive metabolite, Iminoquinone, Danger-associated molecular patterns, Inflammasome