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c-Met Oncogene Enhances Fas-Mediated Apoptosis in B-ALL t(12;21) Cells.

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Abstract

c-Met, the tyrosine-kinase receptor for HGF, has been related to cell motility, proliferation and protection from apoptosis in epithelial cancers (*Cytokine Growth Factor Rev* 2002; 13:41). On the other hand it has been proposed that c-Met could also stimulate apoptosis: association with the pro-apoptotic protein FAS has been shown in some cell types (*Biochem Biophys Res Commun* 1997;230:89). So far no data are available on a conceivable role of c-Met in pediatric B-ALL.

We observed that c-Met is expressed at higher levels in pediatric t(12;21) B-ALL, that better respond to therapy, and in normal B cells compared to B-ALL t(12;21)-, while the latter leukemias do not differ for Fas expression (*Haematologica* 2004;89:113). Here, we studied c-MET/FAS association in t(12;21) B-ALL, and the role of this complex in drug-induced apoptosis.

We performed IP/WB experiments in REH (B-ALL t(12;21)) cells and in healthy CD19+ cells: for the first time we observed in B cells the co-precipitation of c-MET and FAS.

To investigate if the HGF/c-MET pathway could be activated, we stimulated REH cells with HGF for 48 hours. The phosphorylation of c-MET increased 5 minutes after stimulation, and lasted after 48 hours. In HGF stimulated cells we observed a higher proliferation rate compared to non-stimulated cells (p=0.01). We also observed that the c-MET/FAS complex was preseved during HGF stimulation. These results indicate that HGF stimulation does not disrupt the protein complex, while HGF activates the c-MET signaling pathway despite of FAS binding.

To investigate the role of complex formation on FAS activity, REH cells were stimulated with HGF and then treated with Doxorubicin that acts specifically through the FAS apoptosis pathway. After 24 hours of Doxorubicin treatment, we observed in HGF pretreated cells an apoptotic rate higher than in not-pretreated cells ($p=0.03$). These results indicate that the complex c-MET/FAS does not inhibit c-MET or FAS functions, and importantly show that c-MET activation enhances FAS-mediated apoptosis.

To demonstrate that the higher apoptotic sensibility after HGF stimulation is not related to the increased proliferation rate, we treated cells with L-Asparaginase that does not act through the FAS pathway: we did not find an increase of apoptosis after pre-stimulating c-MET with HGF. This is an important result because it confirms that the higher apoptotic rate observed after Doxorubicin treatment in pre-stimulated cells is directly related to increased activation of the FAS pathway. We also verified that the c-MET/FAS complex remains stable after 24 hours from Doxorubicin treatment in apoptotic cells, and results show that it is even more pronounced in HGF treated cells.

To confirm FAS involvement in Doxorubicin treated cells, we checked the cleavage of Caspase-8: cleavage is more apparent in HGF pre-treated cells, demonstrating that the FAS pathway is more effective in c-MET activated cells. After L-Asparaginase treatment Caspase-8 is not cleaved.

The observation that the activation of the c-MET pathway results in increased FAS mediated apoptosis strongly suggests that the c-MET/FAS complex has functional implications for understanding drug mechanisms. The finding that the c-MET/FAS complex is present in normal B cells and in the leukemia cell line model for patients that better respond to therapy implies that this protein complex may have an important role in cellular homeostasis and in enhanced pharmacological response of tumoral cells during therapy.

Author notes

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