Different types of K-Ras mutations could affect drug sensitivity and tumour behaviour in non-small-cell lung cancer

The RAS/MAPK pathway, together with phosphatidylinositol 3-kinase/Akt (PI3K/AKT), is the major signalling network...
Figure 1. Growth and response to treatment of H1299-derived clones expressing different K-Ras mutations. Panel A: in vitro growth of NCI-H1299/K-Ras clone 4 expressing a wild type K-Ras (black dot), NCI-H1299/K-Ras G12C clone 2 (red triangle), NCI-H1299/K-Ras G12D clone 2 (green triangle) and NCI-H1299/K-Ras G12V clone 9 (blue square) cells. Cells were seeded in six well plates and counted every 24 hours. Values represent the mean of three samples ±SD. Panel B: Representative western blot reporting the comparable expression of exogenous K-Ras in the isolated clones. Actin was used as loading control. Panel C: Growth inhibition assay: response of NCI-H1299/K-Ras clone 4 (expressing a wild type K-Ras, black dot), NCI-H1299/K-Ras G12C clone 2 (red triangle), NCI-H1299/K-Ras G12D clone 2 (green triangle) and NCI-H1299/K-Ras G12V clone 9 (blue square) cells to treatment with cisplatin, pemetrexed, gemcitabine, taxol, erlotinib, and sorafenib. Cells were seeded in 96 well plates and grown one day in free drugs medium. Treatments were performed for 2h (cisplatin) or 72h (all other drugs) at the indicated drug concentrations. At the end of the treatment (96 hours after plating cells), the vitality of the cells was assessed by MTS assay (Promega, Italy) following manufacturer’s instructions. The data of the survival curves were plotted as percentages of untreated controls. Each experiment consisted of at least six replicates for each point and the plotted data represent the average mean ±SD of three independent experiments. On the graph are reported the IC50 calculated for each clones on specific drug. Both growth and response to treatment experiments were performed in two independent clones isolated for each specific K-Ras mutant. The figure reports the results obtained in one of the clones for each mutant for simplicity. The two independent clones of each mutant had anyway a similar growth and response to treatment.
linking epidermal growth factor receptor (EGFR) activation to cell proliferation and survival.

Recently, Janakiraman et al. [1] reported evidence that genetic K-Ras alterations beyond the classical ones could have an important role in human cancer. They suggested that different Ras alleles could have non-identical biological activities, which could be important for the selection of the therapy.

While K-Ras mutations seem to negatively select patients affected with colon cancer [2] for EGFR inhibitors treatment, their role in non-small-cell lung cancer (NSCLC) is still debated [3, 4].

We are conducting a phase III randomised controlled trial (TAILOR, NCT00637910), comparing erlotinib to docetaxel in second line, aimed at evaluating the final role of all biological features in predicting efficacy to treatment in EGFR non-mutated patients. Up to date, we have prospectively collected a total of 434 samples of patients affected with NSCLC. In 99 patients out of 434 (22.8%) tumours, a mutation of K-Ras was found. At least nine kinds of mutations were identified according to the replaced basis or amino acid substitution (G12C 39%, G12V 21.8%, G12D 15.6%, G12A 9.3%, G12S 1.5%, G13D 6.2%, G13C 3.1%, G12R 1.5% and G12F 1.5%). In particular, we found that G12C is the most expressed mutation in lung cancer, while in colon cancer, G12D is the most common one as shown in the Sanger Registry (http://www.sanger.ac.uk).

We generated K-Ras-overexpressing clones with the three most common NSCLC amino acid substitutions (G12C, G12V and G12D) from the human NSCLC cell line NCI-H1299 with a wild-type (wt) EGFR. Two independent clones for each mutant were selected. The wt and mutant clones showed a comparable in vitro growth. The expression of a specific K-Ras mutation induces a different sensitivity pattern (Figure 1). In particular, the expression of G12C is associated with a reduced response to cisplatin and an increased sensitivity to taxol and pemetrexed, whereas the expression of G12D mutant resulted in resistance to taxol treatment and sensitivity to sorafenib. The G12V mutant showed a strong sensitivity to cisplatin when compared with the wt clones and was slightly more resistant to pemetrexed. In contrast, the expression of different K-Ras mutants did not modify the cellular response to the EGFR inhibitor erlotinib and to gemcitabine.

In conclusion, our results support the new fascinating hypothesis that different K-Ras mutations may lead to a different signal transduction cascade in NSCLC and to a different carcinogenesis and drug sensitivity. Furthermore, our data suggest that even the replaced amino acids at the same hot spot codon could affect the response.

The data have important clinical implications due to the urgent need of strategies for the treatment of K-Ras–expressing tumours [3, 5] and indicate that the simple definition of K-Ras-mutated tumour could not be enough (without the definition of the specific mutation present) to identify patients with a different probability of responding to therapy in both lung and colon cancers.

disclosure

The authors declare no conflict of interest.

references


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