25P Lung tumorspheres as a drug screening platform against cancer stem cells

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Background: Treatment resistance and metastasis are linked to cancer stem cells (CSCs). This population represents a promising target, but remains unexplored in lung cancer. The main objective of this study was to characterize lung CSCs and discover new therapeutic strategies.

Methods: The study was performed on NSCLC cells from 8 resected patients and 12 cell lines. Suspension cultures (tumorspheres) were established for CSCs enrichment and differentiated tumor cells were cultured as monolayers (2D). The CSCs properties of tumorspheres were assessed in vitro and in vivo. The expression of 60 CSC-related genes was analyzed by RTqPCR and the expression of 12 proteins was evaluated by immunoblot (IB) and immunofluorescence (IF). High-throughput screening was performed using Prestwick and Myria libraries. Selected drugs were administered intraperitoneally to NOD/SCID mice with tumors induced by NSCLC patient and H1650 tumorspheres.

Results: Lung tumorspheres showed unlimited exponential growth (>30 passages), great tumor initiation potential, differentiation capacity, and high resistance to chemotherapy agents, but not to salinomycin. Tumorspheres had significantly higher expression of CSC-related genes (ALDH1A1, KLF4, NANOG, CD44, CD90, CDKN1A, JUNB, MDM2), invasion promoters (MMP9, SNAI1, ITGA6), and Notch (NOTCH1, NOTCH3, DLL4, JAG1) and Wnt (CTNNB1, GSK3B) components than their paired adherent-cultured cells. IB confirmed the overexpression of proteins encoded by CD44, NANOG, CDKN1A, SNAI1, ITGA6, and NOTCH3, and IF showed different localization patterns on lung adenocarcinoma tumorspheres compared with the 2D cultures. Three novel drugs [Disulfiram (DSF), Compound 1 (COMP1) and Compound 2 (COMP2)] with greater cytotoxic potential against lung tumorspheres than monolayer cells were identified. These results were validated in vivo, demonstrating the capacity of these drugs to reduce tumor growth in mice.

Conclusions: Tumorspheres are a useful culture platform for CSCs characterization in a simple and cost-effective way. We found three drugs which are able to diminish the formation and viability of tumorspheres, constituting promising therapies against lung CSCs. Supported by CB16/12/00350, PI12-02838, and PI15-00753 from ISCIII.

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