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The secretome of colon cancer stem cells contains drug-metabolizing enzymes

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ABSTRACT

Drug-resistant cancer stem cells (CSCs) have been implicated in tumor recurrence following chemotherapy. However, the contribution of CSCs to drug-resistance in colorectal cancer is unclear and CSC-intrinsic drug-resistance mechanisms are ill-defined. Here, we address these issues by proteomic analysis of the secretomes of CSCs and isogenic differentiated tumor cells (DTCs) isolated from three distinct metastasized colon tumors.

Mass spectrometry-based proteomics identified 1254 unique proteins in the conditioned media of the paired CSC and DTC cultures. Ingenuity Pathway Analysis revealed that proteins governing 'Cell Death' were most significantly enriched in the CSC secretome. The vast majority of these (37/43) promote cell survival. The CSC secretome is also characterized by a pro-survival Nrf2 antioxidant signature. Interestingly, proteome-maintenance networks are highly enriched in the CSC secretome. CSCs also secrete high levels of drug-metabolizing enzymes, including aldehyde dehydrogenase 1 (ALDH1A1) and bleomycin hydrolase (BLMH). We show that these enzymes cause extracellular detoxification of maphosphamide and bleomycin respectively.

We conclude that colorectal CSCs are characterized by extensive survival and anti-oxidant networks, which are likely to contribute to CSC-intrinsic drug-resistance. In addition, CSCs may modulate drug responses in nearby tumor cells by detoxifying chemotherapeutic drugs in the extracellular space.

Biological significance

Cancer stem cells are thought to play an important role in mediating drug resistance and tumor recurrence following chemotherapy. Therefore, it is important to identify the factors that are secreted by them. Our results provide novel insights into the pathways that govern the intrinsic resistance of CSCs to chemotherapy and, furthermore, demonstrate that they can also inactivate chemotherapeutic drugs in the extracellular space. A better understanding of the pathways that govern drug resistance in CSCs may help in developing effective CSC-targeting drugs.

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1. Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide [1]. In recent years, the prognosis of patients with advanced disease has improved, mainly due to the addition of new targeted drugs to standard treatment regimens [2–5]. However, CRC is characterized by highly divergent tumor responses, and primary and acquired drug resistance is frequently observed.

Colorectal tumors are hierarchically organized with a minor cancer stem cell (CSC) pool driving the formation of more differentiated less malignant cells making up the bulk of the tumor [6–9]. CSCs have also been implicated in drug resistance and tumor recurrence although the nature of this relationship is only beginning to be clarified. Colorectal CSCs display relative resistance to oxaliplatin and 5-fluorouracil [10–12]. We recently identified BIRC6, an Inhibitor of Apoptosis Protein (IAP) as a CSC-intrinsic factor that contributes to resistance against platinum drugs [12]. Likewise, CSCs from other tumor types appear to be generally drug resistant [13]. Treatment of mice bearing colorectal tumor xenografts can cause a dramatic enrichment of CSCs in post-therapy tumor tissue [10,14]. Likewise, post-treatment tumors in breast cancer patients are also characterized by high CSC numbers, suggesting selective CSC-intrinsic drug resistance [15,16]. Nevertheless, the extent of chemotherapy-induced 'selection' for CSCs varies dramatically between studies, indicating that other mechanisms must play a role. Indeed, resistance of colorectal tumors to Irinotecan is mediated by differentiated cells expressing the drug efflux pump ABCB1 [17]. This keeps intra-tumor drug concentrations low and allows CSCs and non-CSCs to survive treatment.

Embryonic SCs contain several genome protection mechanisms, including high expression of DNA repair and anti-oxidant enzymes [18]. Breast CSCs also express high levels of anti-oxidant enzymes that help prevent DNA damage and consequent CSC 'exhaustion' [19]. Furthermore, multiple (C)SC populations, including colorectal CSCs, are characterized by a low proliferation rate, which limits replication-associated DNA damage [20]. Normal SCs of the intestine are an exception as they are actively cycling.

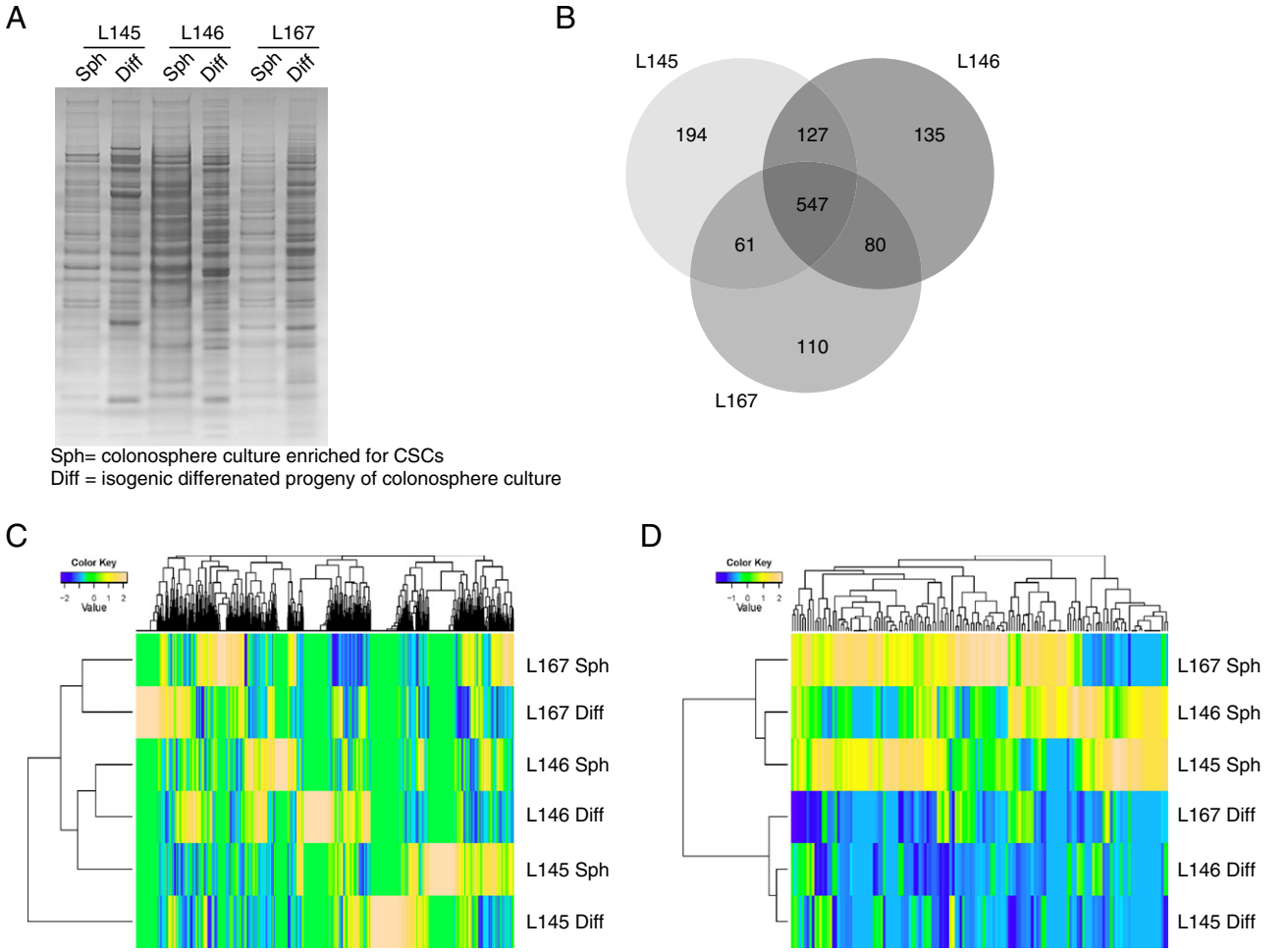

Fig. 1 – Secretome analysis of CSC-cultures and isogenic differentiated tumor cells. (A.) Coomassie-stained protein gradient gel loaded with protein samples from CSC enriched cultures (Sph), and differentiated tumor cells (Diff) from three distinct colorectal liver metastases: L145, L146 and L167. This gel was used for mass-spectrometry analysis. (B.) Venn diagram of all proteins detected in CSC cultures and differentiated TCs. (C.) Heatmap of unsupervised cluster analysis of all identified proteins. (D.) Heatmap of supervised cluster analysis, only including proteins enriched in CSC cultures (Table 1 and Supplemental Table 1) and differentiated cultures.