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Metabolic reprogramming in transformed mouse cortical astrocytes: A proteomic study

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ABSTRACT

Metabolic reprogramming is thought to play a key role in sustaining the survival and proliferation of cancer cells. These changes facilitate for example the uptake and release of nutrients required for nucleotide, protein and lipid synthesis necessary for macromolecule assembly and tumor growth. We applied a 2D-DIGE (two-dimensional differential in-gel electrophoresis) quantitative proteomic analysis to characterize the proteomes of mouse astrocytes that underwent in vitro cancerous transformation, and of their normal counterparts. Metabolic reprogramming effects on enzymatic and structural protein expression as well as associated metabolites abundance were quantified. Using enzymatic activity measurements and zymography, we documented and confirmed several changes in abundance and activity of various isoenzymes likely to participate in metabolic reprogramming. We found that after transformation, the cells increase their expression of glycolytic enzymes, thus augmenting their ability to use aerobic glycolysis (Warburg effect). An increased capacity to dispose of reducing equivalents through lactate production was also documented. Major effects on carbohydrates, amino acids and nucleotides metabolic enzymes were also observed. Conversely, the transformed cells reduced their enzymatic capacity for reactions of tricarboxylic acid oxidation, for neurotransmitter (glutamate) metabolism, for oxidative stress defense and their expression of astroglial markers.

Biological significance
The use of a global approach based on a 2D DIGE analysis allows obtaining a comprehensive view of the metabolic reprogramming undergone by astrocytes upon cancerous transformation. Indeed, except for a few enzymes such as pyruvate carboxylase and glutaminase that were not detected in our initial analysis, pertinent information on the abundance of most enzymes belonging to pathways relevant to metabolic reprogramming was directly obtained. In this *in vitro* model, transformation causes major losses of astrocyte-specific proteins and functions and the acquisition of metabolic adaptations that favor intermediate metabolites production for increased macromolecule biosynthesis. Thus our approach

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appears to be readily applicable for the investigation of changes in protein abundance that determine various transformed cell phenotypes. It could similarly be applied to the evaluation of the effects of treatments aimed at correcting the consequences of cell transformation.

1. Introduction

Major metabolic changes occur in cancer cells. The resulting metabolic reprogramming promotes the synthesis of macromolecules and favors cell survival and tumor proliferation (for reviews see [1,2]). The most frequently described aspect of cancer metabolic reprogramming is the "Warburg effect" also known as aerobic glycolysis [3–5]. This metabolic landmark of tumors is primarily reflected by an increase in glycolytic activity to levels that fulfill the energy needs of cancer cells while maintaining the production of intermediate metabolites required for support of macromolecule biosynthesis. The metabolic reprogramming of cancer cells also often involves an increase in the uptake and metabolization of glutamine that allows the replenishment of tricarboxylic acid cycle (TCA cycle) intermediate metabolites in mitochondria, thus promoting anaplerosis [6]. It also includes a range of enzymatic, proteinic or genetic adaptations that further allow cancer cells to generate a favorable microenvironment for their survival and growth [7–10].

Some of these metabolic peculiarities are not exclusive of cancer cells. The Warburg effect is notably also encountered in normal cells proliferating rapidly [1] as well as in subtypes of quiescent normal cells, such as astrocytes [11]. Of note, astrocytes are one of the brain cell types that can give birth to high-grade gliomas, the most common form of primary brain tumors in adults, and the most aggressive one (for a review see [12,13]).

Like glioma cells, normal astrocytes, even in the presence of a normal oxygen concentration, heavily rely on glycolysis for energy production and eventually release lactate, either from glucose obtained from the vasculature or from internal stores of glycogen [11]. Those stores can be mobilized by various neuroactive signals reflecting neuronal activity [14]. Furthermore, astrocytes actively pump the neurotransmitter glutamate around excitatory synapses and convert this signal of neuronal activity into accelerated glycolysis, producing lactate that is released as fuel for energy production by neurons [15]. In this context, another important metabolic activity of astrocytes is the recycling of glutamate, either by conversion into glutamine through the activity of glutamine synthetase (GS), an enzyme exclusively present in astrocytes [16], or by oxidation via the TCA cycle.

Thus the physiological behavior of astrocytes relies on metabolic specificities similar to glioma cells, namely aerobic glycolysis and a capacity for glutamate/glutamine interconversion. However, the extent of these metabolic adaptations differs between astrocytes and glioma. For example, a recently described method of FRET-based measurement of lactate levels in single cells allowed to calculate a "Warburg effect" ratio between the basal rate of lactate production in the presence of oxygen and the rate of lactate accumulation following inhibition of mitochondrial respiration with azide, the latter reflecting mitochondrial OXPHOS activity [17]. This "Warburg effect" ratio was almost 60-fold higher for a glioma cell line than for primary astrocytes, suggesting a more intense glycolytic activity in the transformed cells and a true defect into mitochondrial function that brings about an extensive metabolic reprogramming. The extent to which metabolic adaptations modify major cell functions is thus an important parameter to evaluate malignancy and characterize different types of cancer and cell lineage in tumors. Although understanding the molecular basis of cancer metabolic reprogramming is an area of intense research, we lack a comprehensive picture of the modifications in metabolic enzymes occurring upon cancerous transformation.

We therefore characterized in a comparative manner the proteomes of astrocytes and of their transformed counterparts using 2D-DIGE analysis [18], coupled with mass spectrometry (MALDI TOF MS/MS). We took advantage of previously described high-grade glioma-initiating cells that were derived from normal astrocytes (NA) of the mouse cortex. Astrocyte transformation was achieved with gamma-irradiation following a de-differentiation step driven by sustained activation of the EGFR signaling pathway [19]. The cells display all canonical features of cancerous transformation, immortalization, uncontrolled growth, cytogenetic alterations, and give rise to high-grade gliomas when injected into nude mice. In culture, they constitute a homogenous population of transformed astrocytes (TA) particularly suited for quantitative biochemical comparison with their normal counterparts, represented by primary cultures of astrocytes. This approach allowed to establish, for the first time, a comprehensive picture of the modifications in nature and relative abundance of many enzymatic species that occur when astrocytes are subjected to tumoral transformation, and to deduce their functional consequences on the cell metabolism.

2. Materials and methods

2.1. Cell culture preparation

Cultures of mouse normal astrocytes were prepared from cortices of 1–2-day-old C57Bl6/J mice according to protocols described by Sharif in 2007 [20] and Prévôt in 2005 [21] with slight adaptations. Briefly, cultures were established in MEM medium (Gibco, Life-technologies, UK) containing 10% fetal calf serum (FCS) (Lonza, BioWhittaker®, Belgium). Medium was changed every 2 days following washes with ice-cold phosphate-buffered saline (PBS, pH 7.4). When confluence was reached, cultures were shaken overnight (250 r.p.m.), trypsinized and seeded in 8 cm dishes at a density of 50,000 cell/cm^2. When confluence was reached again, the cells were maintained in serum-free MEM medium for 48 h, rinsed twice in PBS, and either extracted for protein sample preparation or