A MESSAGE EMERGING FROM DEVELOPMENT: THE REPRESSION OF MITOCHONDRIAL β-F1-ATPase EXPRESSION IN CANCER

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ABSTRACT

Mitochondrial research has experienced a considerable boost during the last decade because organelle malfunctioning is in the genesis and/or progression of a vast array of human pathologies including cancer. The renaissance of mitochondria in the cancer field has been promoted by two main facts: (i) the molecular and functional integration of mitochondrial bioenergetics with the execution of cell death and (ii) the implementation of ^{18}FDG-PET for imaging and staging of tumors in clinical practice. The latter, represents the *bed-side* translational development of the metabolic hallmark that describes the bioenergetic phenotype of most cancer cells as originally predicted at the beginning of previous century by Otto Warburg. In this minireview we will briefly summarize how the study of energy metabolism during liver development forced our encounter with Warburg's postulates and prompted us to study the mechanisms that regulate the biogenesis of mitochondria in the cancer cell.

KEY WORDS:

Mitochondria, H^+-ATP synthase, Oxidative phosphorylation, Glycolysis, Cancer.

INTRODUCTION

Mitochondria play essential roles in cellular energetic metabolism (Ortega and Cuezva, 2005), the execution of cell death (Wang et al., 2001; Jaattela et al., 2004) and intracellular calcium (Satrústegui et al., 2007) and reactive oxygen species (Brunelle et al., 2005; Kaelin et al., 2005) signaling. Therefore, a growing number of human diseases are nowadays associated to the molecular and/or functional alteration of mitochondria (DiMauro and Schon, 2001; Cuezva et al., 2002; Wallace et al., 2005; Lin and Beal, 2006). Mitochondria are highly dynamic organelles whose morphological

changes are linked to their functionality (Rojo et al., 1998; Okamoto and Shaw, 2005; Santamaría et al., 2006; Martínez-Díez et al., 2006). Contrary to a general belief, the different cell types of mammals are endowed with mitochondria that differ significantly in their molecular composition (Mootha et al., 2003). Qualitative and quantitative changes in the ultrastructure, number and function of mitochondria are more evident during development of a given tissue (Valcarce et al., 1988; Izquierdo et al., 1995a; Izquierdo et al., 1995b). In this regard, the study of the cellular and molecular biology of mitochondria in different cell types of mammals, especially in the non-canonical tissues, is urgently required in order to characterize the molecular basis underlying the specific alterations of the mitochondrial proteome observed in different human cancers.

DISCUSSION

The Pasteur Effect in the liver during development. Otto Warburg (Warburg, 1930; Warburg, 1956a and b) first suggested that the high aerobic glycolysis observed in most tumours should result from an impaired bioenergetic function of the mitochondria of the cancer cell. Warburg's formulation was based on the principles of the Pasteur Effect that, in nowadays terminology (Lehninger, 1970), basically states that the metabolic flux of glycolysis in aerobic cells depends on the energy provided in the form of ATP by mitochondrial oxidative phosphorylation. That is, if there is a limitation in the oxygen availability to the cell, or such a cell has a genetic or epigenetic alteration that impairs normal functioning of oxidative phosphorylation, the flux of glycolysis should be increased in order to cope with the cellular energetic demand. Adjustment of the flux of glycolysis could be exerted at short-term by allosteric regulation of key enzymes of the glycolytic pathway and, at long-term, by adjusting the phenotype of the cell through changes in the expression of the enzymes involved in energy generation

pathways. This concept of metabolic regulation illustrated our textbooks of Biochemistry for many years. Nowadays, however, it has faded or disappeared from some of them perhaps because of the same reason why Warburg's hypothesis was neglected (Warburg, 1966; Krebs, 1981) or considered as an epiphenomenon of cancer until recently (Garber, 2004; Garber, 2006).

A beautiful example of the operation of the Pasteur Effect is provided by the rapid shift in the relevance of cellular energy provision pathways (glycolysis *versus* oxidative phosphorylation) of the hepatocytes at the time of mammalian birth (reviewed in Cuezva et al., 1997) (Fig. 1A). During fetal development the hepatocytes derived most of its energy requirements by glycolysis producing large amounts of lactate (Fig. 1A). However, soon after birth, the increased availability of oxygen (Fig. 1A) triggers a sharp reduction in the rates of glucose consumption and so the rates of lactate produced by the neonatal hepatocyte are sharply diminished (Fig. 1A) (Mayor and Cuezva, 1985; Cuezva et al., 1997). The repression of lactate production rates in the neonatal hepatocyte (Fig. 1B) is due to the onset of mitochondrial function and the proliferation of mitochondria (Valcarce et al., 1988; Izquierdo et al., 1995b; Izquierdo et al., 1995a; Cuezva et al., 1997). This example of the switch from glycolysis to oxidative phosphorylation is phenotypically expressed by sharp inverse changes in the relative expression level of the enzymes involved in the energy provision pathways of the liver (Fig. 1B) (Cuezva et al., 1997).

Post-transcriptional regulation of the biogenesis of mitochondria in mammalian liver. The establishment of mitochondrial function in the neonatal liver soon after birth is an active process of mitochondrial biogenesis that requires the coordinated expression of the two genetic systems that encode mitochondrial proteins (Valcarce et al., 1988; Izquierdo et al., 1990; Luis et al., 1993; Izquierdo et al., 1995b).

This process represents the rapid (less than 1h) bioenergetic transformation of pre-existing fetal mitochondria into fully functional organelles (Valcarce et al., 1988; Valcarce et al., 1990; Valcarce and Cuezva, 1991). In contrast with other metabolic pathways that are induced at the same stage of development, the regulation of the expression of both the nuclear and mitochondrial genomes is controlled at post-transcriptional levels (Izquierdo et al., 1990; Luis et al., 1993; Izquierdo et al.,1995b; Ostronoff et al., 1995; Ostronoff et al., 1996). In particular, the mRNAs encoding proteins involved in oxidative phosphorylation accumulated in the fetal liver as a result of developmental changes in the half-life of the corresponding mRNAs (Fig. 2A) (Izquierdo et al., 1995; Ostronoff et al., 1995). However, these accumulated mRNAs are masked, that is, in a translation-repressed state until the time of birth where they become preferential substrates of both the cytosolic and mitochondrial translation machineries (Luis et al., 1993; Izquierdo et al., 1995b; Ostronoff et al., 1996). In the specific case of the mRNA that encodes the catalytic subunit of the H^+-ATP synthase (β-F1-ATPase mRNA), a specific subcellular structure of the hepatocyte controls the localization and cytoplasmic expression of the mRNA (Egea et al., 1997; Ricart et al., 1997; Lithgow et al., 1997; Ricart et al., 2002). Overall, these results illustrated, for the first time in the field of mammalian mitochondrial biogenesis, that processes that control the localization, stability and translation of oxidative phosphorylation genes are relevant for understanding mitochondrial biogenesis during development (Izquierdo et al., 1995b), in cellular proliferation (Martínez-Díez et al., 2006) and in oncogenesis (López de Heredia et al., 2000; Cuezva et al., 2002; Cuezva et al., 2004; Isidoro et al., 2004; Isidoro et al., 2005).

Translational repression of the biogenesis of mitochondria in the cancer cell. Large biochemical similarities exist between tumors and fetal/embryonic tissues. In

fact, these similarities (reviewed in Cuezva et al., 1997) led us to study the mechanisms that regulate the biogenesis of mitochondria and the expression of β-F1-ATPase in rat hepatomas (López de Heredia et al., 2000). We found that mitochondrial biogenesis in hepatomas is repressed when compared to the normal adult liver. The inhibition of β-F1-ATPase expression in As30D and FAO (López de Heredia et al., 2000) as well as in Zadjela (Luciakova and Kuzela, 1992) hepatomas occurs in the paradoxical situation of an increase abundance of β-F1-ATPase mRNA (β-mRNA) (Fig. 2A). Interestingly, the accumulation of oxidative phosphorylation (OXPHOS) transcripts in the hepatomas resulted from an increased stability as compared to the half-life of the transcripts in normal liver (Fig. 2A) (López de Heredia et al., 2000), very much resembling the situation of OXPHOS mRNAs in the fetal liver (Fig. 2A) (Izquierdo et al.,1995; Ostronoff et al., 1995). This further suggested the occurrence of a translational masking event of OXPHOS mRNAs in the cancer cell. Indeed, extracts from rat hepatomas (López de Heredia et al., 2000), as well as from fetal livers (Izquierdo and Cuezva, 1997), exerted a strong and specific inhibition of the synthesis of the precursor protein of β-F1-ATPase in *in vitro* assays. Mechanistically, regulation of β-mRNA translation is explained by differences in the affinity of the mRNA for the components of the translational machinery, as well as by the action of specific proteins that bind regulatory elements within the mRNA preventing their essential role in translation (Fig. 2B) (Izquierdo and Cuezva, 1997; López de Heredia et al., 2000; Ricart et al., 2002). In this regard, the 3'UTR of β-mRNA is essential for efficient translation of the mRNA due to its ability to interact preferentially with components of the translational machinery (Izquierdo and Cuezva, 1997). In fact, the 3'UTR of β-mRNA is endowed with an activity comparable to RNA sites that promote internal initiation of translation (IRES) (Fig. 2B) (Izquierdo and Cuezva, 2000), an activity that is present in certain RNA

sequence elements found in some viral and cellular RNAs (Pelletier and Sonenberg, 1988; Johannes and Sarnow, 1998). That is, functionally the 3' UTR of β-mRNA behaves as a translational enhancer both *in vitro* (Izquierdo and Cuezva, 1997; Izquierdo and Cuezva, 2000) and *in vivo* (Di Liegro et al., 2000). Besides, the control of translation of β-mRNA during liver development (Izquierdo and Cuezva, 1997) and in cancer cells (López de Heredia et al., 2000) has also features in common with other systems. In particular, fetal liver (Izquierdo and Cuezva, 1997; Ricart et al., 2002) and cancer cells (López de Heredia et al., 2000) contain a set of proteins that specifically bind the 3'UTR of the mRNA (Fig. 2B). This binding might sterically hinder ribosome recruitment and the initiation of translation leading to a decreased expression of βF1-ATPase both during fetal development and in liver carcinogenesis (Fig. 2B). Interestingly, the RNA binding activity of these proteins, which display poly A binding specificity (Izquierdo and Cuezva, 2005), is regulated by changes in the cellular redox and energy states (Izquierdo and Cuezva, 2005).

Alteration of the mitochondrial proteome in human tumors: The Bioenergetic Signature of Cancer. The glycolytic phenotype of human tumors has been widely demonstrated at the biochemical, molecular and functional levels (Semenza et al., 2001; Ziegler et al., 2001; Sasaki et al., 2005). However, the alteration of the bioenergetic function of mitochondria in the cancer cell (for a detailed review see Pedersen, 1978) within the context of the original Warburg hypothesis was not formally addressed until recently (Cuezva et al., 2002; Cuezva, et al.,2004; Isidoro et al., 2004; Isidoro et al., 2005). For this purpose, we studied in normal and tumor biopsies derived from the same patients the changes in the expression level of bioenergetic (βF1-ATPase) and structural (hsp60) mitochondrial proteins concurrently with the expression of glycolytic (GAPDH, PK, LDH, HK) markers of the cell. This basic immunological

approach allows a proteomic estimation of both the bioenergetic competence of the organelle (as assessed by the βF1/hsp60 ratio) and of the overall mitochondrial potential of the cell (βF1/hsp60/GAPDH ratio), the two factors that could define the cellular bioenergetic activity of mitochondria. The latter ratio was defined as the Bioenergetic Cellular Index (BEC index) (Cuezva et al., 2002). The results obtained strongly supported the original Warburg's hypothesis since it was observed that in most human carcinomas there was a reduction of the bioenergetic competence of the organelle (drop in the βF1/hsp60 ratio) concurrent with the up-regulation of glycolytic (GAPDH) markers. This results in a sharp reduction of the BEC index of the tumor when compared to the normal tissue (Cuezva et al., 2002; Cuezva, et al., 2004; Isidoro et al., 2004; Isidoro et al., 2005). These findings have been confirmed and extended to other carcinomas (Unwin et al., 2003; Meierhofer et al., 2004; Yin et al., 2004; Hervouet et al., 2005; He et al., 2004; Mazzanti et al., 2006). In fact, almost all tumor samples analyzed within each type of neoplasia showed the alteration of the BEC index, further providing a marker of diagnostic applicability with a sensitivity > 97% (Cuezva et al., 2004; Isidoro et al., 2005). These are the main reasons why the down-regulation of the BEC index of the tumors has been named as the *Bioenergetic Signature of Cancer.*

The existence of a common feature to most types of carcinomas led us to consider the bioenergetic signature as a tool for the diagnosis and prognosis of cancer patients. Indeed, we should emphasize that the Bioenergetic Signature of Cancer significantly correlates with the survival of colon (Cuezva et al.,. 2002), lung (Cuezva et al., 2004), and breast (Isidoro et al., 2005) cancer patients, strongly suggesting the implication of mitochondria in cancer progression. Furthermore, the bioenergetic signature of the tumors has been further suggested to provide a predictive marker of the

response of the tumor to chemotherapy (Shin et al., 2005) and for the design of future strategies in cancer treatment (Santamaria et al., 2006; Tomiyama et al., 2006).

Several interpretations have been provided aimed at explaining the Warburg effect in tumors (Osthus et al., 2000; Semenza et al., 2001; Cuezva et al., 2002; Govindarajan et al., 2005; Garber, 2006; Matoba et al., 2006). Some authors suggest that the shift to a glycolytic phenotype results from tumor adaptation to the hypoxic environment where the tumor develops (Semenza et al., 2001). Others support that it results from mutations in oncogenes and proteins related to signal transduction pathways (myc, Akt, mTOR) that in turn promote changes in the expression of genes involved in cellular energetic metabolism (Osthus et al., 2000; Rathmell et al., 2003; Schieke et al., 2006; Bensaad et al., 2006). More recently, a direct effect of HIF1α (Selak et al., 2005; Papandreou et al., 2006; Kim et al., 2006; Kim and Dang, 2006) and p53 (Matoba et al., 2006) activities has been suggested to impair mitochondrial bioenergetics. Likewise, other studies have described the occurrence of mutations on mtDNA (Polyak et al., 1998; Fliss et al., 2000; Carew and Huang, 2002; Tan et al., 2002) or in nuclear genes involved in the metabolic and bioenergetic function of the organelle (Baysal et al., 2000; Habano et al., 2003; Neumann et al., 2004). Irrespective of the ultimate genetic and/or epigenetic cause that could explain the Warburg phenotype of the cancer cell we think that translational control of the expression of β-mRNA is a mechanism that contributes to the alteration of the bioenergetic phenotype of human tumors (see Fig. 2B), as was the case in the rat liver during development (Luis et al., 1993; Izquierdo and Cuezva, 1997) and in rat hepatomas (López de Heredia et al., 2000). In fact, both virtual (Lal et al., 1999) and real estimation (http://www.oncomine.org) of the relative cellular expression level of β-mRNA reveals that the transcript is paradoxically up-regulated in most human carcinomas when

compared to the expression found in normal tissues. Therefore, the characterization of the molecular and cellular biology of β-mRNA in different human cell types is a prerequisite in order to understand the altered mitochondrial phenotype of human tumors. In fact, carcinogenesis causes at least two different alterations of the mitochondrial phenotype in the human cancer cell (Fig. 2C) (Cuezva et al., 2002). In liver cells, carcinogenesis involves a depletion of the organelles most likely because the mechanisms that control mitochondrial proliferation are affected (Fig. 2C) (Cuezva et al., 2002). In contrast, in colon, lung and perhaps in other tissues, carcinogenesis specifically affects the expression of β-F1-ATPase suggesting alterations in the mechanisms that control mitochondrial differentiation (Fig. 2C) (Cuezva et al., 2002). Obviously, we cannot exclude the possibility that the decreased expression of β-F1-ATPase observed in cancers could also result from the establishment of an exacerbated degradation of the protein in the cancer cell.

H^+-ATP synthase, cell death and cancer. Increased and decreased cellular mitochondrial activities are respectively associated with suppression (Schulz et al., 2006) and development (Thierbach et al., 2005) of cancer. Moreover, the execution of cell death also requires an efficient oxidative phosphorylation (Dey and Moraes, 2000; Park et al., 2004; Kim et al., 2002; Tomiyama et al., 2006), being specifically required the molecular components of the H^+-ATP synthase (Matsuyama et al., 1998; Matsuyama et al., 2000; Gross et al., 2000; Harris et al., 2000) . In fact, the H^+-ATP synthase is necessary for the efficient execution of apoptosis (Santamaria et al., 2006) in cells that have a high dependence on oxidative phosphorylation for the provision of metabolic energy, thus providing another evidence linking metabolism to cell death (Plas and Thompson, 2002; Danial et al., 2003; Azoulay-Zohar et al., 2004; Vahsen et al., 2004). The role of the H^+-ATP synthase in the execution of cell death is mediated by

controlling the generation of reactive oxygen species which in turn promote a severe oxidative damage on cellular and mitochondrial proteins, favoring in this way the release of apoptogenic molecules from the organelle (Santamaria et al., 2006). In contrast, highly glycolytic cells, with scarce or no dependence on oxidative phosphorylation for energy provision, do not produce reactive oxygen species after the toxic insult and are resistant to mitochondria-geared cell death stimuli (Santamaria et al., 2006). These findings have led us to suggest that repression of the bioenergetic function of mitochondria is a hallmark strategy of the tumor cell in order to ensure its perpetuation.

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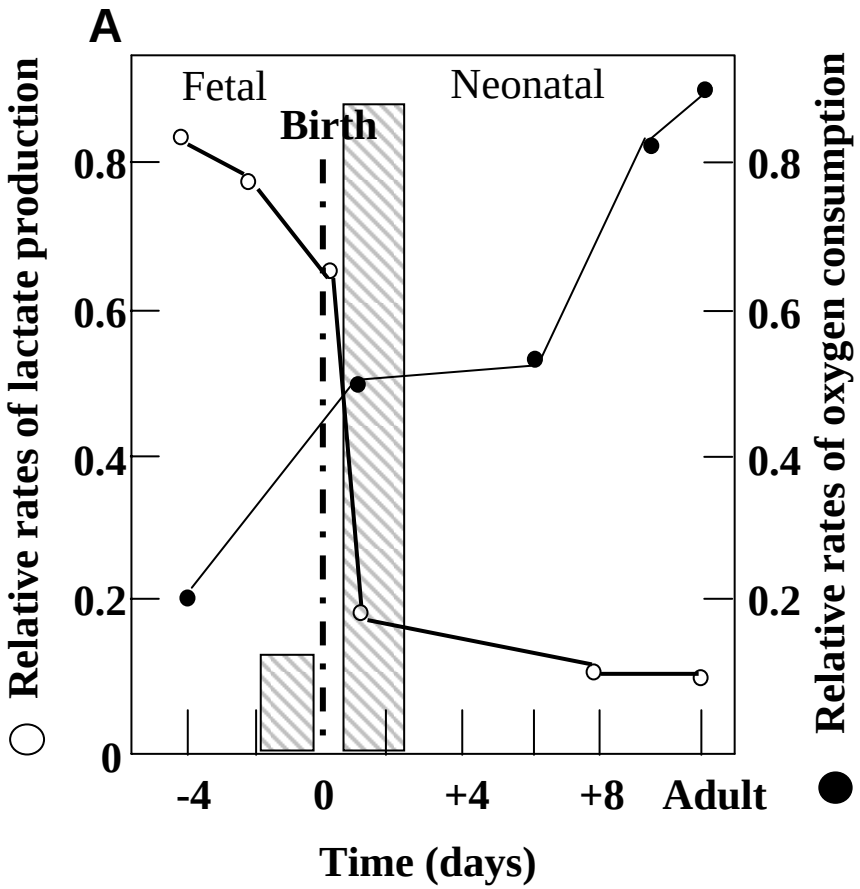
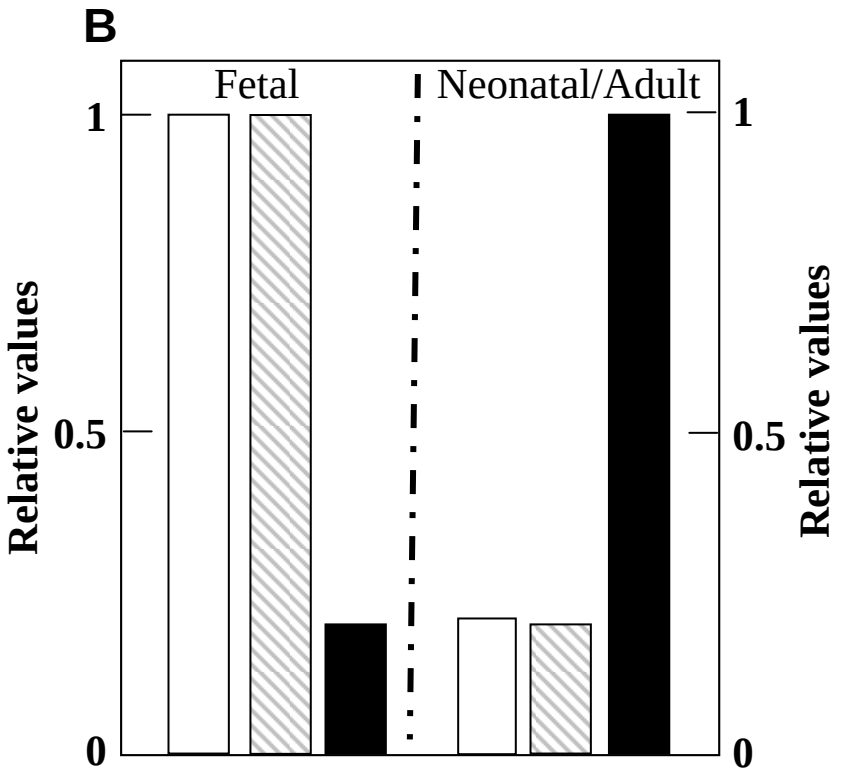
LEGEND TO FIGURES

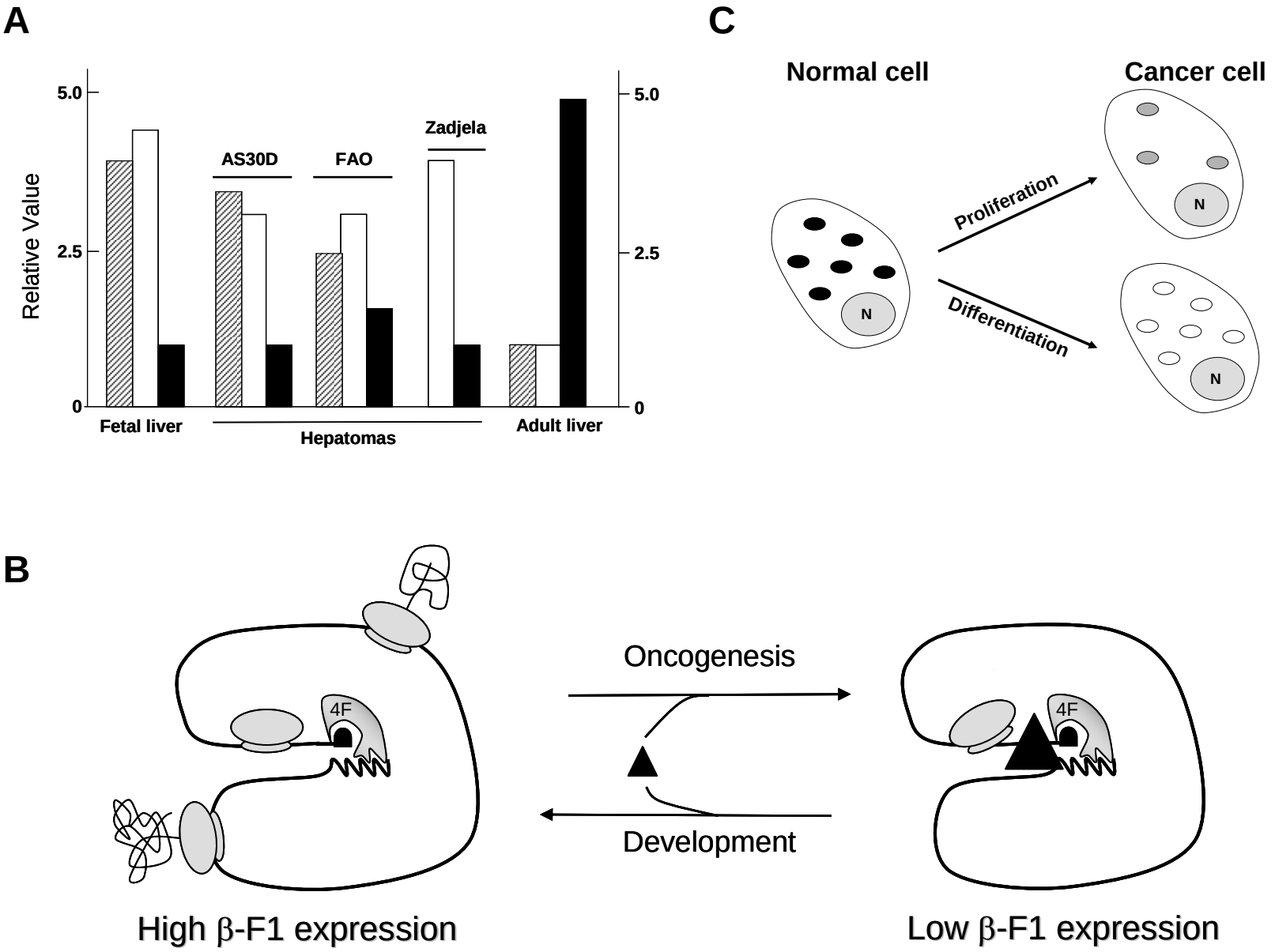
Figure 1. <u>The Pasteur Effect during development of the rat liver.</u> **A,** Birth triggers a profound ten-fold increase in blood oxygen concentrations in the neonatal rat (hatched bars). The rates of aerobic glycolysis (open circles) are sharply diminished after birth (discontinuous line) as a result of the onset of mitochondrial biogenesis in the neonatal hepatocyte. Changes in oxygen consumption rates of the hepatocytes as development of

the liver proceeds are also indicated (closed circles). **B,** Shows the rapid and profound changes in the *in vivo* estimated lactate turnover rates (open bars) and the expression level of glycolytic enzymes (hatched bars) and mitochondrial β-F1-ATPase protein (closed bars) per unit of liver during development of the rat.

Figure 2. <u>Repression of β-F1-ATPase expression in the cancer cell.</u> A, Shows the relative values of β-F1-ATPase mRNA turnover (hatched bars), mRNA expression (open bars) and β-F1-ATPase protein (closed bars) assessed at different stages of liver development of the rat and in three rat hepatomas (AS30D, FAO, Zadjela). **B,** The diagram illustrates two states (High and Low Efficiency) of the translation of β-F1-ATPase mRNA (black line). High β-F1 expression is achieved when there is no activity of proteins that bind the 3'UTR of the mRNA (Neonatal/Adult liver). Low β-F1 expression (Fetal liver/Hepatomas) results from the binding of β-mRNABPs (closed triangles) to the 3'UTR of the mRNA, hampering in this way the efficient initiation of translation of the mRNA. The translation of β-F1-ATPase mRNA is depicted on a circular molecule due to the physical cross-talk of both mRNA ends exerted by the scaffold provided by translation initiation complex eIF4F. Ribosomes and nascent polypeptides are also represented. **C,** The alteration of mitochondrial phenotype of the cancer cell could result from interference in the program of organelle proliferation and/or differentiation, in both cases the BEC index is reduced.


A
Fetal
Neonatal
Birth
○ Relative rates of lactate production
● Relative rates of oxygen consumption
0
0.2
0.4
0.6
0.8
-4
0
+4
+8
Adult
Time (days)


B
Fetal
Neonatal/Adult
Relative values
0
0.5
1


A
5.0
2.5
0
Relative Value
AS30D
FAO
Zadjela
Fetal liver
Hepatomas
Adult liver
C
Normal cell
Cancer cell
N
Proliferation
Differentiation
B
4F
Oncogenesis
Development
High β-F1 expression
Low β-F1 expression