

Research & Reviews on Cervical Cancer

Chapter 3

Uterine Cervix Cancer Cells Re-Establish the Natural Lactate Rich Microenviron- ment, favoring Disease Progression

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1. Metabolic Fitness is Crucial for Cancer Initiation and Progression.

The way a cell undergoes malignant transformation should meet their capacity of surviving in the microenvironment of the organ where the cancer will develop. Metabolic adaptation is for sure one of the criteria that must be accomplished, driven by metabolic plasticity that allows the adaptation of cancer cells to the availability of energy and biomass sources that will sustain cell survival and proliferation. Each human organ has a particular microenvironment which is created by several cell types and in some cases also by symbiotic microorganisms. These biological partners are constantly sharing organic compounds and signaling molecules that will control cell proliferation and differentiation, accounting for the organ's function.

Uterine cervix has a particular acidic microenvironment created by the biochemical collaboration of epithelial cells and symbiotic bacteria, mainly *Lactobacillus sp.* This microenvironment constitutes a selective challenge for cancer cells that are more prone to carry out the progression of the disease. The metabolic dynamics of glucose versus lactate is particularly important in this context, and this chapter will present some data showing the relevance of cancer cells in the maintenance of the lactate rich microenvironment.

2. Uterine Cervix Histology

The uterine cervix has two components: the ectocervix that protrudes into vagina and the endocervix which is a canal that links the vagina to the uterus. The endocervical canal is lined by cells similar to endometrium which covers the uterine cavity – a single layer of

tall, columnar, mucus-secreting cells. The ectocervix is directly exposed to the hostile acidic microenvironment of the vagina, being lined by a thick stratified squamous epithelium [1,2]. Physiologically, the endocervical columnar epithelium in the portion more close to ectocervix undergoes metaplastic transformation to a mature squamous epithelium, being this transformation especially active during adolescence and pregnancy [1]. The metaplastic dynamics in this transformation zone occurs throughout female reproductive life, and it is regulated by environmental stressors such as hormones and pH [3]. Although squamous metaplasia of the cervix are physiological, the newly differentiated squamous epithelium of the transformation zone is vulnerable to cell injury and damage [3], having an increased risk of oncogenesis, principally through the infection of Human Papillomavirus (HPV) [1,2], which are the main etiological factor for uterine cervix cancer [4].

3. Microflora Contributes for the Metabolic Environment

It has been known for a long time that Lactobacilli are the predominant microorganisms found in the cervix and vagina, together with some skin and fecal contaminants [5,6]. In 1861, Albert Döderlein described the physiology of vaginal and cervix microbiota, considering *Lactobacillus* genus the most prevalent group of bacteria which since then received his name Döderlein Bacilli [7]. Despite a controversial study claims that new microbial isolation and identification techniques show that often *Lactobacillus* are not the predominant genus in vaginal/cervical microflora [8], several other publications until today corroborate Döderlein's findings [5,9-13].

As mentioned, the uterine cervix has two main portions the ecto and the endocervix. The ectocervix is externally coated with a squamous stratified epithelium whereas the endocervix exhibits a glandular columnar epithelium. The squamous cells, from vagina and ectocervix, are full of glycogen and upon the physiological peeling process the cell disintegration allows the glycogen release into microenvironment. This glycogen is the main source of food for Döderlein bacilli, glycogen is degraded at first into glucose and afterwards, through lactic fermentation, glucose gives rise to lactic acid (lactate). Lactic acid is a very important player in the control of chemical and physical conditions, being the main responsible for the acidification of uterine cervix and vaginal microenvironment [14,15] (**Figure 1**). It has been known for decades that cancer cells arising from the squamous mucosa of the cervix and vagina lose the ability to accumulate glycogen [16]. This fact undoubtedly demonstrates the metabolic remodelling of cancer cells.

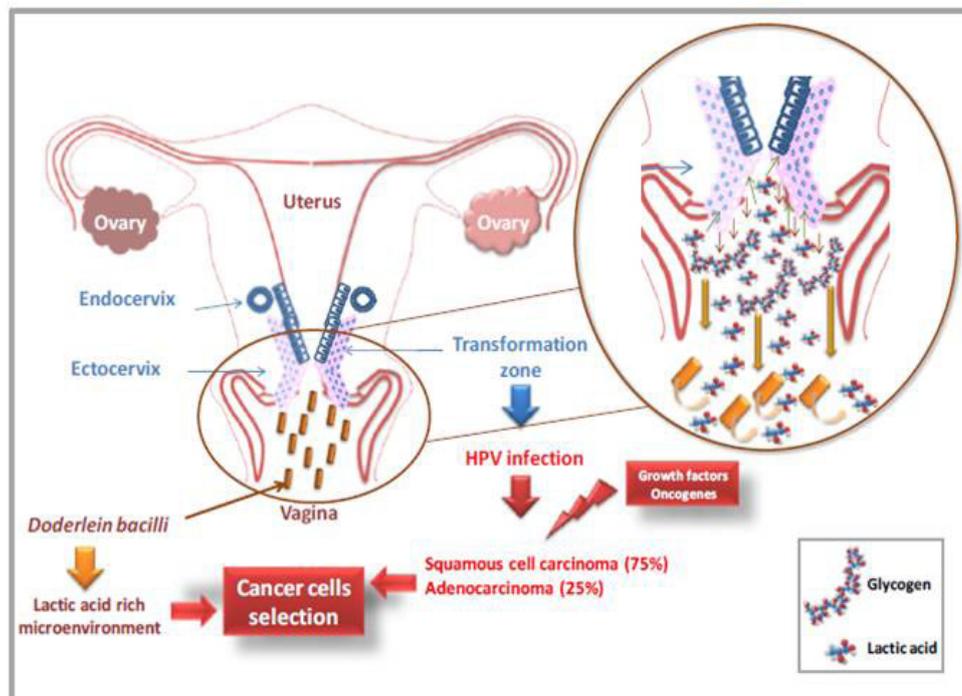


Figure 1. Natural metabolic symbiosis between uterine cervix squamous cells and symbiotic flora.

The squamous cells, from vagina and ectocervix, are full of glycogen and upon the physiological peeling process the cell disintegration allows the glycogen release into microenvironment. Döderlein bacilli degrade glycogen at first into glucose and then into lactic acid through lactic fermentation. Lactic acid is the main responsible for the acidification of uterine cervix and vaginal microenvironment that can constitute a selective factor for cancer cells that are more able to carry cancer progression. Human Papillomavirus (HPV) infects the metaplastic (transformation) zone and it is the main etiological factor for uterine cervix carcinogenesis, which is driven by the activation of oncogenes and signaling regulators and mediators.

4. Lactate versus Glucose Metabolism in Cancer

The most well documented metabolic adaptation in cancer is called the Warburg effect and it defines the glycolysis that occurs independently of the oxygen levels - aerobic glycolysis - and that was for several decades considered as a common adaptation of all the cancer types. There is some debate about the selective advantages that glycolytic metabolism provides to proliferating tumor cells. Initial works pointed that tumor cells develop defects in mitochondrial function and that aerobic glycolysis is a necessary adaptation to the lack of ATP production through oxidative phosphorylation [17]. However, it was later appreciated that mitochondrial defects are rare [18] and tumors retain the capacity of oxidative phosphorylation and consume oxygen at rates similar to those observed in normal tissues [19,20]. Because the energetic yield of glycolysis is much lower than cellular respiration, glycolysis would occur at a very high rate, having the lactate production as a final event.

Alternatively, it has been proposed that glycolytic metabolism arises as an adaptation to hypoxic conditions during the early avascular phase of tumor development, as it allows for ATP production in the absence of oxygen. Adaptation to the resulting acidified microenvironment that is caused by excessive lactate production may further drive the evolution of the glycolytic phenotype [21,22]. So, at first lactate was considered an excretion product of glucose

accelerated metabolism, however today it is well known that lactate can also be a carbon and energy source [23-26].

Most recently, it has been proposed that aerobic glycolysis provides a biosynthetic advantage for tumor cells, and that a high flux of substrate through glycolysis allows for effective shunting of carbon to key subsidiary biosynthetic pathways [27]. Moreover, glycolysis wouldn't be a predominantly energetic source but a biomass supplying source. Our group have some studies [28], also in uterine cervix [26], showing that glycolysis mainly supplies pentose-phosphate pathway (PPP) for the synthesis of nucleotides that will support cell proliferation.

Recently, our group has shown that glucose and lactate metabolic profiles are dependent on the histological type of uterine cervix carcinomas. Squamous cell carcinoma, originated from ectocervix cells, is predominantly a lactate consumer; cells are able to uptake lactate and convert it into pyruvate and Krebs cycle intermediates as well as into amino acids and fatty acids. Adenocarcinoma cells, originated from endocervix glandular cells, exhibit a glycolytic phenotype; they are almost exclusively lactate producers, using glucose as a source [26]. This observation is again consistent with the fact that squamous ectocervix cells are naturally more exposed and adaptable to lactic acid metabolism than glandular endocervix cells.

The lactate enrichment of cancer microenvironment is as consequence of the high rate of glycolysis that must occur in tumors that rely on glucose as an energetic coin. In tumors that have lactate as an energetic source and glucose as a biomass source, lactate must be transiently secreted in order to avoid cell damage due to intracellular acidity and to be further taken up according to cell energy and biomass demands. Uterine cervix cancer is an excellent model of how the retention by cancer cells of features resembling the natural counterparts is an advantage for cancer to survive in the natural organ's microenvironment.

The natural uterine cervix microenvironment is rich in lactate produced by Döderlein bacilli from epithelial glycogen; in malignant neoplasms, cancer cells decrease the production of glycogen as they increase their glucose demands but at the same time they produce high levels of lactate that will keep the acidity of microenvironment. This way cancer cells keep the metabolic favorable conditions of microenvironment that will positively select cancer cells that are more prone to go on with cancer progression (**Figure 2**). Furthermore, because the symbiosis between cancer cells and Lactobacilli is replaced by symbiosis between cancer cells, the bacterial density decreases in cancerous uterine cervix (**Figure 2**). Moreover, the pro-apoptotic effect exerted by Lactobacilli on cancer cells [29] is also depleted by all this microenvironmental metabolic remodeling.

Of course all of this orchestrated metabolic circuit mainly depends on the expression profile of monocarboxylate transporters (MCTs) and lactate dehydrogenases (LDHs).

5. Dynamics of Monocarboxylate Transporters (MCTs) and Lactate Dehydrogenases (LDHs) Expression

Metabolic fitness of cancer cells to a certain microenvironment implies the modulation of the expression of several intervenients. The dual metabolism glucose versus lactate is dependent on a tightly regulated expression of glucose transporters to supply glycolysis, lactate dehydrogenases that will catalyze the interconversion of pyruvate and lactate and monocarboxylate transporters that will mediate the export and import of lactate. As the metabolic adaptation is a very important cancer hallmark [30,31] all the players (transporters and enzymes) are often pointed out as suitable targets to fight cancer.

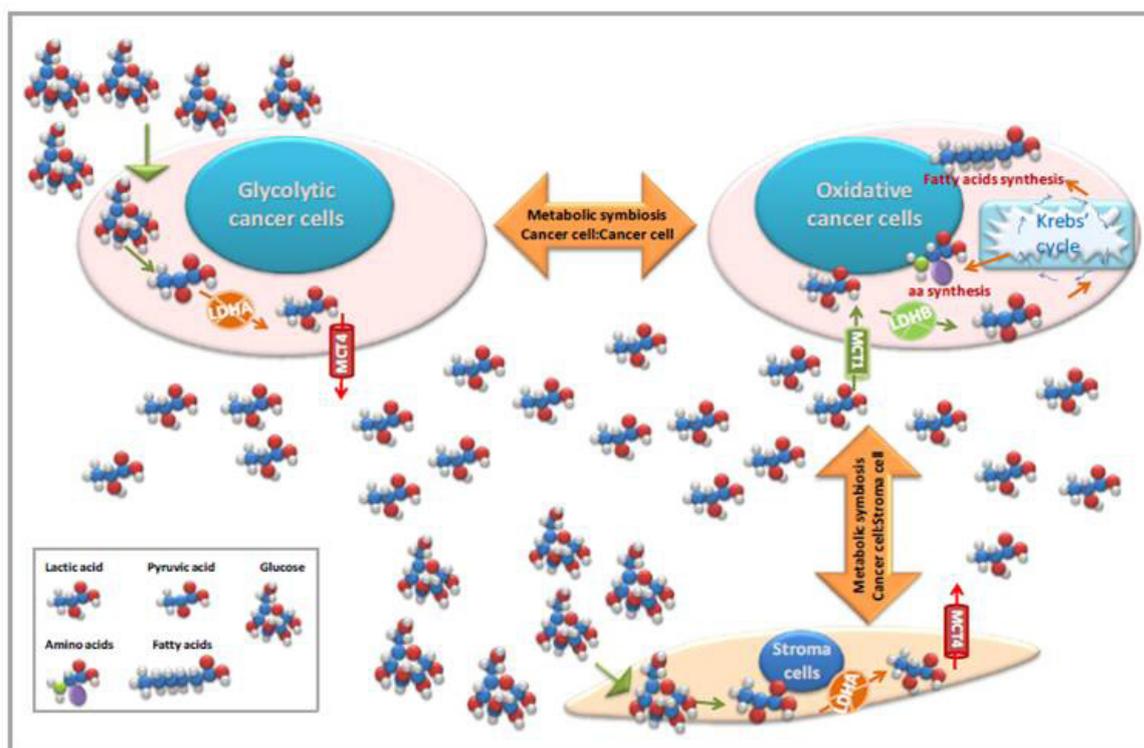


Figure 2. Metabolic symbiosis between cancer cells and between cancer cells and stroma cells.

In malignant neoplasms, cancer cells decrease the production of glycogen as they increase their glucose demands but at the same time they produce high levels of lactate that will keep the acidity of microenvironment. Decreased levels of glycogen decreases the presence of symbiotic bacteria, therefore a metabolic symbiosis is established between glycolytic cancer cells and oxidative cancer cells, which are respectively able to produce and consume lactate. This symbiosis can also occur between oxidative cancer cells and normal glycolytic stromal cells that will serve as lactate suppliers to be consumed by cancer cells. This way cancer keeps the metabolic acidic conditions of microenvironment that are favorable to cancer progression.

Lactate dehydrogenases (LDHs) catalyze the reversible pyruvate reduction into lactate and it also allows glycolytic cells to maintain the levels of pyruvate low enough to avoid cell death due to pyruvate cytotoxic events as an alkylating agent [32,33]. LDHs work as tetramers of two different subunits: subunit LDH-H is encoded by the LDHB gene and is ubiquitously expressed in healthy tissues, whereas subunit LDH-M is encoded by LDHA gene [34]. Compared to LDH-M, LDH-H has a higher K_m for pyruvate and a higher V_{max} for pyruvate reduction [35], being more prone to convert lactate into pyruvate than the opposite. Consequently, LDH5/LDH-4M (LDHA gene) preferentially catalyzes the reduction of pyruvate into lactate

and plays key roles in the maintenance of a high glycolytic flux and in resistance to apoptosis. Elevated LDH5 expression is of unfavorable prognostic significance in many human tumors [36,37]. Conversely, LDH1/LDH-4H (LDHB gene) expression is most commonly silenced in glycolytic cancer cells, a process involving hypermethylation of the promoter of LDHB gene [32,38]. Nevertheless, enzymatic redundancy of LDHs accounts for metabolic functioning of cancer cells as shown by the maintenance of glycolysis and lactate production capacity of cancer cells upon exogenous silencing of LDHA [39].

In cancer context, MCT1 (SLC16A1 gene) and MCT4 (SLC16A3 gene) passive lactate–proton symporters [40] are the most relevant [25,41,42]. Besides they are chemically able to mediate both import and export of lactate, in cancer there is a coordination and MCT1 is more frequently associated to the import and MCT4 to the export of lactate [23,25,26,43,44]. Biochemical differences between MCT1 and MCT4 define the roles they play in cancer. MCT1 shows a higher affinity for lactate than MCT4 but MCT4 has a higher turnover rate than MCT1 [45]. So, MCT1 are more prone to mediate lactate entry into oxidative cancer cells and MCT4 mediates the export of lactate in glycolytic cancer cells and stromal normal cells [45,46].

The expression of these two transporters allows the establishment of a metabolic symbiosis between different cancer cells and between cancer cells and normal stromal cells (**Figure 2**), as some cells can produce lactate to be consumed by other cells sharing the same niche [23,43,47-52].

According to the main roles in cancer of MCT1, MCT4 and LDHA and LDHB encoded enzymes, metabolic reasonable but not totally restricted partnerships can be advanced, MCT4 works coordinated with LDHA encoded enzymes in cancer cells with high glycolytic phenotype and MCT1 is a partner of LDHB related enzymes in cancer cells with an oxidative phenotype [23,43] and our team have shown this in uterine cervix cancer which is a natural microenvironment suitable to stimulate both metabolic routes [26] (**Figure 3**). However, both metabolic phenotypes can co-exist in the same cancer cell. In our experience in uterine cervix cancer, some squamous cell carcinoma cells exhibit a dual address profile of lactate production and consume, again showing that this cell type survival and disease progression benefits of the maintenance of lactic acid rich cervix microenvironment.

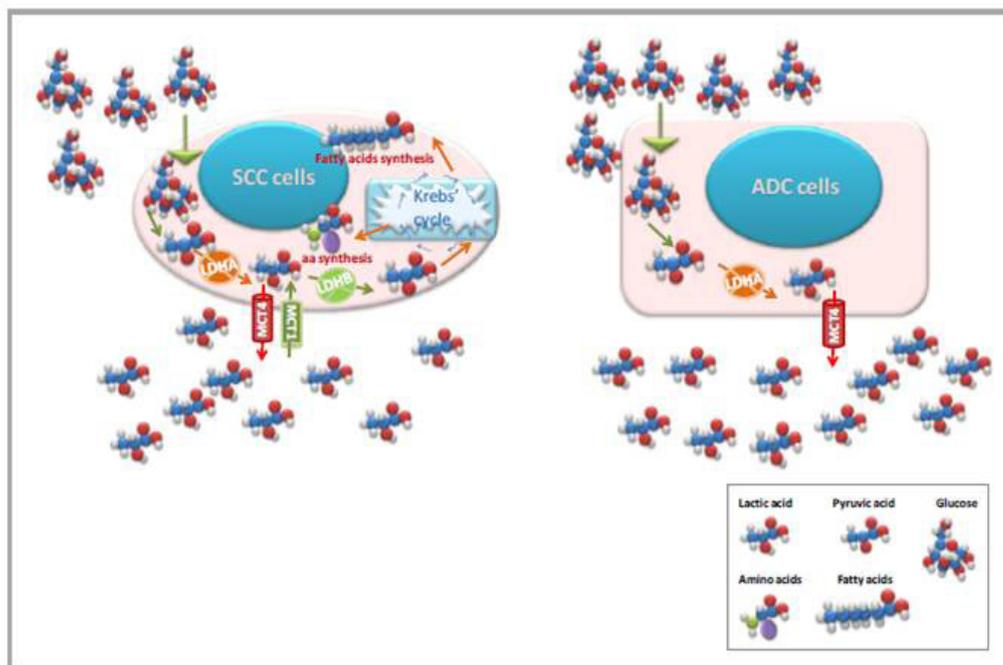


Figure 3. Metabolic profiles of uterine cervix cancer cells depends on the histological type.

Squamous cell carcinoma, originated from ectocervix cells, is predominantly a lactate consumer; cells are able to uptake lactate and convert it into pyruvate and Krebs' cycle intermediates as well as into amino acids and fatty acids. Adenocarcinoma cells, originated from endocervix glandular cells, exhibit a glycolytic phenotype; they are almost exclusively lactate producers, using glucose as a source. Often squamous cells consume both lactate and glucose, so lactate must be transiently secreted to be further taken up according to the cell demands on energy and biomass.

6. Regulation of Metabolic Remodeling

Metabolic remodeling in cancer is a direct consequence of energy and biomass demands to support cell proliferation, allowing tumor growth and disease progression. Metabolic switch is not required to be supported by a brand new built cancer specific network of signaling and metabolic routes. Cancer metabolic remodeling is essentially an adjustment of metabolic flux according to cancer cells demands to survive and proliferate in a certain microenvironment; being regulated by the main mitogenic and pro-survival signaling pathways [53,54] as presented in **Figure 4**.

For sure, the cancer cell histological origin and the organ microenvironment, in which a tumor will develop, are determinant selective prerogatives for cancer metabolic remodeling and fitness of each specific cancer type. In uterine cervix cancer, Epidermal growth factor (EGF) seems to be the main growth factor acting on the stimulation of cancer progression [55]. EGF activates RAS dependent pathways through the stimulation of tyrosine kinase receptors (RTK), being EGFR the main receptor playing on cervical cancer [56,57]. PI3K/AKT/mTOR is also a Ras dependent pathway [58], which together with STAT3, have been recently identified as the main responsible for the control of uterine cervix cancer cells proliferation [55,56]. Hence, it would be expected that the main intervenients in lactate metabolism (LDHA/B and MCT1/4) ought to be regulated by these signaling pathways (**Figure 4**).

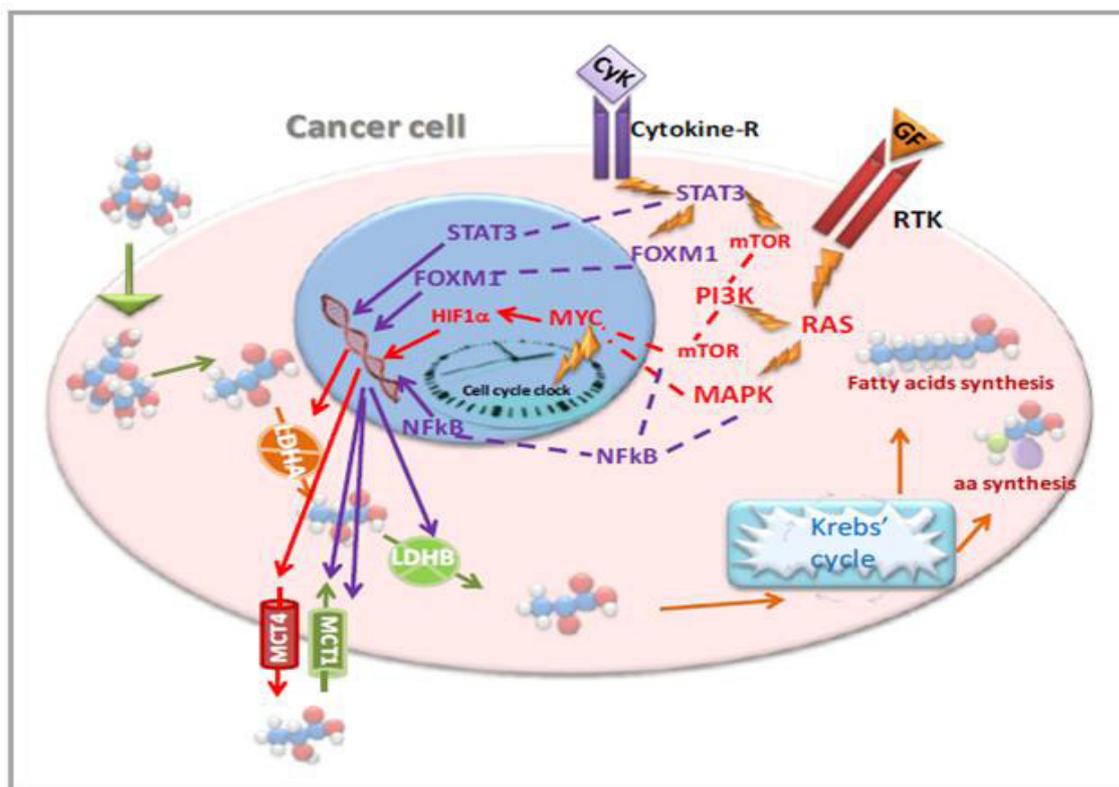


Figure 4. Main mitogenic pathways regulate metabolic adaptation in uterine cervix cancer.

Growth factors (GF) activate RAS dependent pathways through the stimulation of tyrosine kinase receptors (RTK). Activated RAS activates MAPK and PI3K/AKT/mTOR pathways, which together with STAT3 activated by cytokines, are the main responsible for the control of uterine cervix cancer cells proliferation and metabolic remodeling. MAPK and PI3K/AKT/mTOR pathways activate Myc and HIF1 α that will regulate the expression of LDHA and MCT4. STAT3 directly will activate LDHB expression and through the activation of FOXM1 it will activate MCT1. STAT3 can be activated by cytokines or by PI3K/AKT/mTOR pathway. MCT1 can also be expressed under the action of NF κ B activated by MAPK and PI3K/AKT/mTOR pathways and not by the canonical TNF α signaling.

The expression of LDHA and MCT1 is regulated by the well known oncogene c-Myc [54,59-61], which expression and activity is regulated by the main mitogenic Ras dependent pathways [58]. In hypoxia c-Myc cooperates with HIF1 α to regulate LDHA expression [60,62,63], and HIF1 α also regulates MCT4 expression [64] in a PI3K/mTOR dependent manner [65]. In various cancer models, it was demonstrated that the signal transducer and activator of transcription 3 (STAT3) regulates LDHB gene expression [26,66]. STAT3 is canonically activated by cytokine receptors (JAK:STAT pathway) [56,67] but it can also be activated by Src and Ras dependent pathways [68–70]. The TNF α pathway effector NF κ B, which can also be activated by Ras and PI3K dependent pathways [58], is an important player in MCT1 regulation in cancer cells metabolic remodeling and survival [71] and in cancer: stroma cells metabolic cross-talk [48]. NF κ B was identified as pivotal in cancer angiogenesis promotion in a context of metabolic collaboration between cancer and endothelial cells in which MCT1 and MCT4 are key elements [48]. This dynamics involves interleukin 8 (IL-8) which is also an activator of JAK: STAT pathway, and it was shown that IL-8 targeting would prevent lactate induced angiogenesis [48]. Our team observed in uterine cervix cancer that the expression of both MCT1 and LDHB partners in lactate consumer cells was dependent on STAT3 action.

LDHB was regulated by direct binding of STAT3 to its promoter and MCT1 expression was regulated by FOXM1, which activation is dependent on STAT3 [26,72-74]. Our results were corroborated with findings in human cervix cancer specimens in which FOXM1 associates with MCT1 expression in squamous cell carcinomas and FOXO3a [75] (repressor of FOXM1 target genes) with MCT4 expression in adenocarcinomas [26].

7. Highlightings

- A natural metabolic symbiosis works on between epithelial cells and symbiotic bacteria. In this biochemical cross-talk, epithelial squamous cells release glycogen that is metabolized by Lactobacilli in order to produce lactic acid, which will maintain the acidity of microenvironment.
- The high glucose demands of uterine cervix cancer cells prompt these cells to lose the ability to accumulate glycogen, which limits the number of symbiotic bacteria in the microenvironment. The metabolic loop is restored by the establishment of metabolic symbiosis between metabolically different cancer cells (glycolytic versus oxidative) and between oxidative cancer cells and normal glycolytic stromal cells.
- These metabolic remodeling is orchestrated by the same signaling pathways that command carcinogenesis and cancer progression.

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