**FINAL DEGREE PROJECT**

# MELANOGENESIS INTERFERENCE BY INTRACELLULAR BACTERIA: EXPLORING A PATHOPHYSIOLOGICAL MECHANISM IN CANCER (MELANOMA)

**María Rosselló Herrera**

**Degree in Medicine**

**Faculty of Medicine**

**Academic Year 2022-23**

# MELANOGENESIS INTERFERENCE BY INTRACELLULAR BACTERIA: EXPLORING A PATHOPHYSIOLOGICAL MECHANISM IN CANCER (MELANOMA)

**María Rosselló Herrera**

**Bachelor's Thesis**

**Faculty of Medicine**

**University of the Balearic Islands**

**Academic Year 2022-23**

Keywords:

Intracellular bacteria, host-pathogen interaction, intracellular signaling, tumorigenic transformation, melanoma, melanomagenesis, melanin synthesis.

*Thesis Supervisor's Name: Ramon Llull Cerdà*

# Abstract

An association between bacteria and tumorigenesis has been long established. Recent studies have identified intracellular bacteria within cancer cells, including melanoma, although its role in carcinogenesis remains unclear. In the context of melanoma, it can be speculated whether intracellular bacteria could interfere with the host cell signaling pathways and, consequently, contribute to melanoma development. In this review, the main signaling pathways in melanomagenesis and melanogenesis are summarized and correlated with intracellular bacteria-induced mechanisms that could trigger or influence tumorigenesis at different levels. Although several mechanisms were compatible, the evidence so far has identified *Bartonella henselae*, *Fusobacterium nucleatum*, *Mycoplasma hyorhinis*, and *Staphylococcus aureus* within melanoma cells. It remains a hypothesis whether these bacteria could be involved in melanomagenesis. At present, no pro-melanoma interference between these intracellular bacteria and the eukaryotic niche has been demonstrated *per se*; thus, further studies are needed.

# Index

# 1. Introduction and Objectives

Classically, bacterial interference with the host cell has been restricted to the extracellular environment. However, increasing evidence supports that some bacteria are able to survive within the host cell, allowing a close interaction between a prokaryotic organism and the eukaryotic niche, which could lead to the deregulation of crucial host cell pathways that may trigger pro-tumorigenic mechanisms.

In 2020, Nejman et al. characterized the human tumor microbiome and demonstrated that intratumor bacteria were predominantly intracellular. Moreover, in a recent study, Kalaora et al. reported the presentation of intracellular bacteria-derived peptides in human leukocyte antigens (HLA) in melanoma cells. The role of melanocytes in the immune skin response has been described, which might suggest a more frequent interaction with bacteria (and, therefore, intracellular bacteria). In this context, it has been hypothesized that intracellular bacteria are more than bystanders and therefore, could contribute to melanomagenesis in varying degrees.

Melanoma develops from the malignant transformation of melanocytes and constitutes the most aggressive type of skin cancer. Over the past decades, an increase in its incidence has been reported, mainly in developed countries. Identifying an association between intracellular bacteria and melanoma development has the potential to change our current understanding of not only melanomagenesis but also tumorigenesis, and establish a new approach to cancer treatment.

This bachelor's thesis aims to review, on the one hand, the evidence regarding the presence of intracellular bacteria in tumor cells and the tumorigenic interference with the host cell and, on the other hand, the evidence concerning melanomagenesis and melanogenesis. Based on this data, the involvement of intracellular bacteria-driven mechanisms in melanomagenesis will be hypothesized.

## 2. Methodology

Initial bibliographic research focused on the interaction between the host cell and intracellular bacteria was conducted in PubMed with the following MeSH descriptors: "bacteria" "cytoplasm", "host-pathogen interactions", "apoptosis", "carcinogenesis", "cell transformation, neoplastic", "neoplasm" and "humans". Subsequently, a second research focused on melanoma and melanogenesis was conducted with the following MeSH descriptors: "bacteria", "microbiota", "melanoma", "signal transduction", "cell transformation, neoplastic" "melanins" and "humans". The obtained literature was assessed through the abstract or full text.

## 3. Results

### 3.1 Intracellular bacteria

Several bacterial pathogens (as well as other microorganisms such as fungi, protozoa, or viruses) have evolved to invade and survive within a eukaryotic niche (1). A classification regarding its ability to survive and replicate extra or intracellularly distinguishes between facultative (these bacteria are able to replicate within or outside the host cell) or obligate intracellular bacteria (replication is restricted within the intracellular niche) (2).

Some *Bartonella*, *Bordetella*, *Brucella*, *Burkholderia*, *Campylobacter*, *Escherichia*, *Francisella*, *Fusobacterium*, *Helicobacter*, *Legionella*, *Listeria*, *Mycobacterium*, *Neisseria*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, and *Yersinia* species are facultative intracellular bacteria, while some *Chlamydia*, *Coxiella*, *Rickettsia*, and *Mycobacterium* species are obligated intracellular bacteria, among others (1).

Intracellular bacteria have developed multiple strategies to invade, replicate and survive inside the host cell. Different intracellular life cycles have been described, distinguishing between an intravacuolar/intraphagosomal or cytosolic lifestyle (1). Following internalization, endocytic pathways are activated, and intracellular bacteria are incorporated within a membrane-bound vacuole or phagosome, which undergoes progressive acidification and maturation through fusion with endocytic organelles, completed with the development of a phagolysosome (1). Intracellular bacteria with an intravacuolar lifestyle are able to arrest the phagosome maturation process and modify the environment within the phagolysosome (except *Coxiella burnetii*, whose survival is dependent on phagolysosome formation) (1,3). Some of these pathogens hijack the host endocytic and secretory pathways to establish a specialized niche for bacterial growth, replication, and survival (3).

However, some pathogens have developed mechanisms to escape from the vacuole into the nutrient-rich host cytosol, which must be permissive for bacterial growth; in turn, bacteria must be able to use cytosolic substrates. Furthermore, intracellular bacteria with a cytosolic lifestyle must avoid microbicidal substances and modulate the host defense mechanisms (through evasion of immune recognition, inhibition of the immune and inflammatory response, and suppression of autophagy) (4).

Pathogens are able to interact with other intracellular compartments within the host cell to ensure its survival (for instance, the endoplasmic reticulum, Golgi-related vesicles, mitochondria, or the eukaryotic nucleus) without altering host cell integrity (2).

In this context, multiple studies have demonstrated its ability to remain viable intracellularly without engaging in the destruction of the host cell; in fact, intracellular bacteria could actively promote evasion of host cell death via activation and inhibition of pro-survival and pro-death pathways, respectively (1,5). For instance, a study demonstrated that *Bordetella burgdorferi* was able to invade human neuroglial and cortical neuronal cells; bacterial viability within the host cell and the absence of cytopathic effects following infection were also reported (6).

As stated above, intracellular bacteria can survive within the host cell through interaction and deregulation of its cellular pathways.

## 3.2 Intracellular bacteria in tumor cells

Replication and survival of intracellular bacteria within several cell types (which include phagocytic and non-phagocytic cells) have been reported (1).

Increasing evidence supports the existence of a human intratumor microbiome, which could influence tumorigenesis through several mechanisms (7). Recently, an analysis of the human tumor microbiome in samples of seven solid tumor types (including melanoma) demonstrated that intratumor bacteria were principally intracellular, mainly located inside cancer and immune cells; moreover, intracellular bacteria were present in varying degrees according to the tumor type. In addition to its identification, the authors reported that these pathogens remained viable and active within tumor cells (8). However, it is unclear whether these bacteria could contribute to tumorigenesis or whether they solely exploit the tumor environment.

Nevertheless, bacteria and cancer are two interrelated terms. Identification of bacterial deoxyribonucleic acid (DNA) integration in cancer cell genomes and upregulation of ancient genes or conserved genes in primitive and unicellular

organisms as a common feature in tumors, suggest that cancer may represent a return to a unicellular lifestyle (9). In consonance with this evidence, a hypothesis regarding the bacterial origin of cancer cells has been proposed, based on a prokaryote-to-eukaryote transition in which intracellular bacteria may play a decisive role (9).

According to this hypothesis (9), following senescence, normal or cancer cells transform into giant polyploid cells or giant polyploid cancer cells, respectively, which promote the activation of "latent" intracellular bacteria and allow the internalization of extracellular bacteria. Subsequently, intracellular bacteria may translocate into the nuclei, acquire and retain nuclear DNA in DNA storage bodies, and transform into nascent cancer cells, which proliferate within the cytoplasm and later are released. As the nascent cancer cell develops, disruption of the DNA storage bodies (which allows hybridization between eukaryotic DNA and bacterial DNA) and further changes may contribute to the development of primary or secondary cancer cells.

To summarize, evidence describes intracellular bacteria within tumor cells and allows speculation about a crucial role in tumorigenesis, although its extent remains unexplored.

## 3.3    Intracellular bacteria's potential to induce tumorigenesis

Accumulating evidence regarding the role of intracellular bacteria in carcinogenesis strongly suggests that the localization of the microorganism within the host cell could enable and promote several cellular pathways that would remain inaccessible or that would require more complex mechanisms to extracellular bacteria.

Some intracellular bacteria have been associated with specific tumors; however, others induce mechanisms that are potentially associated with cancer development, but a link with a specific cancer type has not been established yet. Some of these mechanisms do not require the presence of bacteria within the host cell but are crucial to allow internalization.

*Chlamydia*, an obligate intracellular bacteria, has evolved to manipulate host cell pathways to ensure a replicative niche: alterations in gene expression and protein production at multiple levels being a common outcome. Evidence regarding cervical cancer supports several mechanisms by which *Chlamydia trachomatis* could contribute to tumorigenesis, such as induction of genomic instability and DNA damage, inhibition of mitochondrial apoptosis, activation of oncogenic pathways (the mitogen-activated protein kinase/extracellular signal-related kinase or MAPK/ERK pathway), promotion of invasion, metastasis and a pro-inflammatory environment, among others (10). Similarly, *Chlamydia*

*pneumoniae* infection may be implicated in lung cancer tumorigenesis; the promotion of an inflammatory environment, anti-apoptotic activity, and the secretion of mutagenic metabolites are some of the proposed mechanisms, although evidence remains unclear (11).

Another obligate intracellular bacteria, **Coxiella burnetii**, which may be involved in B-cell non-Hodgkin lymphoma development (12), modulates several hallmarks of cancer cells, such as the regulation of host cell apoptosis. At a transcriptional level, *Coxiella* manipulates the expression of survival-related genes (the expression of pro-survival genes and pro-apoptosis genes increased and decreased, respectively) (13). Moreover, interference between autophagy-related Beclin-1 and anti-apoptotic Bcl-2 interaction and several effector proteins harbored by *Coxiella burnetii* also contribute to the anti-apoptotic effect. This outcome requires bacterial protein synthesis (13), suggesting that intracellular bacteria play an active role, instead of remaining as bystanders.

The contribution of **Helicobacter pylori** to gastric cancer has been established. In relation to intracellular bacteria, studies have detected this pathogen within gastric pre-neoplastic and neoplastic cells. The intracellular expression of virulence genes such as the vacuolating cytotoxin (vacA) and cytotoxin-associated gene A (cagA) may promote a major involvement in gastric carcinogenesis; for instance, cagA-mediated activation of oncogene protein tyrosine phosphatase non-receptor type 11 (*PTPN11*), subsequent *PTPN11*-encoded protein tyrosine phosphatase 2 (SHP2) and signal transducer and activator of transcription 3 (STAT3) (14,15). Moreover, a study demonstrated that *Helicobacter pylori*, through cagA and overexpression of capping actin protein of muscle Z-line alpha subunit 1 (CAPZA1), might inhibit the protective effect of autophagy in the host cell against tumorigenesis and intracellular bacteria growth, thus increasing the risk of gastric carcinogenesis (15).

**Salmonella enterica** might be involved in gallbladder cancer pathogenesis since it has been reported to induce malignant transformation of genetically susceptible cells. *Salmonella*-mediated translocation of bacterial effector proteins into the host cell, critical to enhance internalization and intracellular survival, is responsible for sustained activation of MAPK and protein kinase B (AKT) signaling. Considering that the transformation state persists after bacterial eradication, it has been suggested that constitutive MAPK and AKT activation may alter the host transcriptome; therefore, pathogens could be major drivers of epigenetic changes (16).

*Salmonella* infection has also been associated with colorectal carcinoma. AvrA, a bacterial effector protein, may play a role in colonic tumorigenesis since a study reported that tumor incidence in mice infected with *Salmonella* expressing AvrA was higher in comparison to those with AvrA-deficient bacteria (17). In addition to

its contribution to the establishment of an intracellular niche (18), this effector protein is also implicated in apoptosis inhibition and the activation of several components associated with cancer development (such as STAT3) (17).

A link between **Escherichia coli** and colorectal cancer has been proposed: *Escherichia coli* colonizing colorectal cancer has been found to survive and replicate within human macrophages, leading to the secretion of pro-inflammatory cytokines. Therefore, intracellular bacteria may modulate carcinogenesis by interacting with immune cells (19).

Interaction between **Mycobacterium tuberculosis** and the host cell may be implicated in lung cancer tumorigenesis. The persistence of intracellular bacteria might lead to a malignant transformation and cancer progression in the infected host cell. For instance, a study reported *Mycobacterium tuberculosis* stimulated tumor cell proliferation and migration through overexpression of marker of proliferation Ki-67 (*MKI67*) (20). Moreover, this bacteria is able to invade tumor-infiltrating immune cells (macrophages), promoting a pro-inflammatory environment and epithelial-to-mesenchymal transition (21).

**Staphylococcus** species contribute to carcinogenesis in several types of cancer, such as breast, bladder, colon, liver, lung, oral, and skin cancer, as well as glioblastoma and lymphoma. Some mechanisms promoted by intracellular bacteria might be involved in tumorigenesis. *Staphylococcus aureus* may induce a reactive oxygen species (ROS)-dependent genotoxic effect (22) and upregulate the MAPK/ERK signaling pathway; moreover, through type VII secretion system effectors, this bacteria can directly manipulate cell death pathways in order to suppress apoptosis and preserve the integrity of the niche (23).

Extensive evidence sustains the role of **Fusobacterium nucleatum** in colorectal carcinogenesis. The abundance of *Fusobacterium nucleatum* is reportedly increased from normal tissues to adenoma and adenocarcinoma tissues (and intracellular colonization has been demonstrated) (24). Moreover, this bacteria enhances the proliferative and invasive potential of colorectal cell lines (24), corroborating its contribution to colorectal cancer development. In this context, several *Fusobacterium*-induced mechanisms have been proposed, such as activation of pro-oncogenic signaling (for instance, via activation of β-catenin signaling, which is also implicated in DNA damage), non-coding RNA (involved in oncogenesis and metabolic reprogramming) and induction of a pro-inflammatory tumor-favorable immune environment, among others (25). Although the activation of some of these mechanisms requires virulent factors involved in adhesion and invasion of the host cell, a study has demonstrated that invasion is not required for tumor growth (26).

Its contribution to the development of other tumor types (breast, bladder, cervical, esophageal, gastric, head and neck, lung, and pancreatic cancer) is controversial.

## 3.4    The case of melanoma

### 3.4.1 Melanomagenesis

Melanoma pathogenesis, that is, melanomagenesis, involves multiple signaling pathways (27).

Melanoma has the highest mutational burden of any cancer. Several **mutated driver genes** have been identified, including *BRAF*, *NRAS*, *NF1*, *KIT*, *PTEN*, *CDKN2A*, *TERT*, and *TP53*, which are responsible for the hyperactivation of the MAPK (RAS/RAF/MEK/ERK pathway, regulated by *BRAF*, *NRAS*, *NF1*, and *KIT*), PI3K/AKT (regulated by *PTEN*), cell-cycle regulation (regulated by *CDKN2A*), p53 (regulated by *TP53*) and pigmentation-related pathways, among others (27).

Interference with **key transcriptional factors** and their **downstream signal pathways** contributes to melanocytes' acquisition of malignant attributes: SOX10 (which regulates cell proliferation and migration), microphthalmia-associated transcription factor (MITF) (which modulates the expression of genes implicated in cell-cycle regulation, cell differentiation, and invasiveness), Notch (which regulates cell migration and several oncogenic signaling pathways such as the MAPK or β-catenin pathway) and Wnt-β-catenin (evidence is controversial: both a pro-tumoral and tumor-suppressive role have been proposed) (27).

In particular, MITF regulates melanocyte development and homeostasis, contributing to the modulation of proliferation, differentiation, survival, and pigmentation. Its biological function highly correlates with its activity level. On the one hand, low MITF expression has been associated with enhanced invasiveness of melanoma cells since downregulation of MITF may induce the activation of STAT3 (a transcription factor involved in multiple phases of melanomagenesis). On the other hand, moderate MITF expression facilitates a proliferative state through the activation of several pro-survival targets (27).

**Epigenetic** modifications and deregulation are also implicated in melanoma pathogenesis. For instance, di or tri-methylation of histone 3 on lysine-4 on regulatory sites proximal to genes involved in specific modulation of oncogenic pathways in melanoma have been reported (27).

In melanoma (and other cancer types) development, **metabolic reprogramming** is fundamental. Predominantly, it involves the preference for aerobic glycolysis instead of mitochondrial oxidative phosphorylation (termed the "Warburg effect"). Lipid (including enhanced lipogenesis and lipid uptake) and amino acid metabolism deregulation were also displayed in melanoma (27).

**Cell-adhesion alteration**, **epithelial-mesenchymal translation** (EMT), and **exosomes** play crucial roles in tumor metastasis. Specifically, EMT is a process whereby epithelial cells adopt a mesenchymal phenotype and display increased migratory and invasive behavior (27).

**Inflammatory factors** and **signal pathways** are involved in carcinogenesis. In particular, constitutive activation of inflammasomes could induce an increased interleukin-1β (IL-1β) secretion, which is involved in melanoma development and progression (through angiogenesis and modulation of immune cells) (27). Moreover, **angiogenic growth factors**, such as vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) also contribute to melanomagenesis (27).

The role of **melanogenesis** and **ROS** in melanoma will be discussed below. Regarding the latter, it has been demonstrated that melanoma cells produce higher levels of ROS in comparison with non-tumoral cells. These highly reactive molecules promote oncogenic mutations and crucial signaling pathways involved in cell proliferation, differentiation, and dissemination (28).

### 3.4.2 Melanogenesis in melanomagenesis

#### 3.4.2.1 Regulation of melanogenesis

Melanin biosynthesis occurs in melanosomes within melanocytes. Tyrosinase and tyrosinase-related proteins 1 and 2, whose expression is regulated by **MITF** (29), are key enzymes in the regulation of the multiple catalytic reactions implicated in melanogenesis, that is, the conversion of an amino acid (tyrosine) to melanin (eumelanin and pheomelanin).

Several pathways have been involved in the regulation of MITF expression and melanogenesis.

Alpha-melanocyte-stimulating hormone (α-MSH) stimulates the melanocortin receptor 1 (MC1R) and activates protein kinase A (PKA); PKA induces phosphorylation of the cAMP response element-binding protein (CREB), which results in MITF upregulation (29).

The **MAPK** pathway is also implicated in MITF regulation; however, evidence regarding the MAPK signaling pathway, including activation of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 kinase, is controversial. On the one hand, activation of ERK1/ERK2 induces MITF phosphorylation and promotes its degradation, thus downregulating melanogenesis (29). On the other hand, ERK, JNK, and p38 phosphorylation could upregulate MITF expression and transcriptional activity (30).

Activation of the **PI3K/AKT** pathway induces glycogen synthase kinase-3 beta (GSk3β) phosphorylation and subsequent MITF degradation, which leads to melanogenesis downregulation (29). In contrast, stimulation of the Wnt pathway suppresses GSk3β phosphorylation and β-catenin degradation. Following cytoplasmic accumulation, **β-catenin** translocates into the nucleus and binds to the lymphoid-enhancing factor/T-cell factor (LEF/TCF) transcription factors, with subsequent upregulation of MITF expression (29).

The nuclear factor kappa-light-chain-enhancer of activated B cells (**NF-κB**) signaling pathway and **p53** are also responsible for melanogenesis upregulation (30,31). Furthermore, increased levels of ROS (which could associate DNA damage, NF-κB, and p53 activation) may lead to a similar effect. However, ROS-ERK stimulation due to mitochondrial dynamics could decrease melanin synthesis (31).

**Mitochondrial dynamics** modulate melanin biosynthesis in human epidermal melanocytes with varying results; while mitochondrial fusion increased melanin synthesis, mitochondrial fission reduced melanogenesis through activation of the ERK pathway and subsequent degradation of MITF (32).

**Inflammation** mediators could also influence this process; for instance, several cytokines downregulate the expression of melanogenesis-related genes (31).

### 3.4.2.2 Melanogenesis and melanomagenesis

The role of **melanin** and **melanogenesis** in melanoma is controversial. For instance, melanin (through ultraviolet radiation absorption) protects melanocytes; also, its presence inhibits melanoma metastasis. However, a positive association between melanin and melanomagenesis can not be ruled out since melanogenesis constitutes a source of intracellular ROS. Moreover, melanin levels positively correlate with the concentration of ROS within the cell (33).

Depending on its concentration, **ROS** may favor tumor progression (moderate ROS levels) or suppression (excessive ROS accumulation). As described above, ROS accumulation has been associated with mutagenic and genotoxic effects. Furthermore, ROS are involved in the induction and activation of oncogenes (such as *BRAF*) and transcription factors, including activator protein-1 (AP-1), hypoxia-inducible factor (HIF-1), NF-κB, or STAT3; the MAPK/ERK and PI3K/AKT signaling pathways; and metabolic reprogramming (33,34). All these mechanisms reportedly lead to melanoma.

In this context, many cross-points are shared between the genetic regulation processes of melanogenesis, cell proliferation, and inhibition of apoptosis.

Moreover, numerous mutations in key nodes of melanogenesis regulation pathways have been identified in melanoma (35).

In conclusion, melanogenesis interference may be involved in tumorigenesis.

### 3.4.3 Intracellular bacteria in melanoma

Intracellular bacteria have been identified in several tumor types, including melanoma. An analysis of 17 melanoma metastases demonstrated that multiple bacteria-derived peptides were presented through HLA-I and HLA-II molecules, in both antigen-presenting and melanoma cells (36).

Although the same study demonstrates that tumor-colonizing bacteria can invade melanoma cells and that subsequent bacteria-derived antigen presentation is possible, this evidence neither implies that intracellular bacteria remain viable within the host cell nor proves a prokaryote-eukaryote interference that could lead to melanomagenesis or other deregulations. Among the identified bacteria, *Staphylococcus aureus* and *Fusobacterium nucleatum* (which is one of the most abundant genera in the melanoma microbiome) (7) associate with pro-tumorigenic mechanisms (as discussed below).

Furthermore, *Bartonella henselae*, which has been reported to trigger vascular tumorigenesis, was identified within melanoma cells and might contribute to melanomagenesis through several mechanisms involved in pro-angiogenic signaling (as exposed below) (37,38).

Another study described *Mycoplasma hyorhinis* invasion and survival within melanoma cells (39). *Mycoplasma* infection of a melanoma cell line upregulated genes involved in metabolism, cell cycle, and apoptosis regulation (40); however, no conclusive association between intracellular mechanisms and tumorigenesis has been reported.

### 3.4.4 Potential involvement of intracellular bacteria in melanomagenesis

Possible intracellular bacteria-induced mechanisms and their role in melanomagenesis will be discussed. Although these mechanisms have only been reported in non-melanoma tumors, the possibility of an association between intracellular bacteria-driven interference and melanomagenesis will be discussed and hypothesized.

#### 3.4.4.1   Chromosomal instability and genotoxicity

Several intracellular bacteria may induce **chromosomal instability** and **genotoxicity**, which could increase predisposition to mutations and create an environment favorable to malignant cell transformation. Genome instability and mutations are a hallmark of cancer cells, including melanoma. *Chlamydia*

*trachomatis* promotes chromosomal instability and aneuploidy (10). Moreover, intracellular *Chlamydia trachomatis*, *Propionibacterium acnes*, *Shigella*, and *Staphylococcus aureus* trigger ROS-mediated DNA damage and downregulation of DNA damage repair mechanisms (5,10,22,41). Specifically, *Chlamydia trachomatis* impairs repairing mechanisms (a common feature in melanoma) through inhibition of the recruitment of DNA damage response proteins (such as ataxia telangiectasia-mutated gene product or pATM); loss of pATM expression associates with melanoma progression (42,43).

### 3.4.4.2   Mutation of driver genes and downstream components of signaling pathways

Intracellular bacteria could induce **mutation of driver genes** and **downstream components** of **signaling pathways.**

Proto-oncogene **c-Myc** overexpression in melanoma promotes cell proliferation, migration, invasiveness, and aerobic glycolysis (44). *Salmonella*, through effector AvrA, which has been suggested to mediate bacterial intracellular survival (18), is able to activate β-catenin and upregulate c-Myc expression (45).

Interference with the **cell-cycle regulation pathway** may induce malignant transformation of melanocytes. In particular, Cyclin D1 has been proposed as a melanoma oncogene, and its upregulation (which could be stimulated by some intracellular bacteria) may promote uncontrolled cell proliferation (27). Moreover, *Chlamydia trachomatis* persistent infection could upregulate Cyclin E expression, which may enhance cell proliferation (42); although Cyclin E is overexpressed in melanoma and might contribute to melanomagenesis, evidence is scarce in comparison to Cyclin D1 (27,46).

Inactivation of **p53** has been described in melanoma (47). An analysis of host transcriptional responses upon *Burkholderia cepacia* infection demonstrated the downregulation of the p53 signaling pathway (48). Although *Burkholderia* reportedly invaded and survived within the host cell, a causal link between intracellular localization and p53 modulation was not evidenced (48). However, *CDKN2A* mutations have been proposed as the major responsible for p53 inactivation in melanoma.

**STAT3** is involved in the regulation of cell proliferation, apoptosis, angiogenesis, tumor invasion and metastasis, and the inflammatory response in several tumors, including melanoma (49). STAT3 activation promotes tumor cell proliferation through the deregulation of factors implicated in melanomagenesis, such as upregulation of Cyclin-D1, c-Myc, and anti-apoptotic Bcl-2 members Mcl-1, Bcl-xL, and Bcl-w (17,27).

*Helicobacter pylori* (through intracellular expression of cagA and overproduction of ROS upon infection) (14), *Mycobacterium tuberculosis* (17), *Propionibacterium* (41), and *Salmonella* (through bacterial effector protein AvrA) (18) induce activation of STAT3, which is also crucial for *Mycobacterium tuberculosis* intracellular survival (17).

**β-catenin** is a protein that modulates intracellular signal transduction and regulates the expression of several target genes involved in cell proliferation, survival, and migration (49). The role of β-catenin in melanoma development is controversial (49). Although it has been suggested that it may act as a melanoma suppressor, accumulation of nuclear β-catenin could activate TCF/LEF transcription factors and induce the expression of Cyclin D1, MITF, or c-Myc (among others), all of them implicated in melanomagenesis (27).

Evidence supports that *Salmonella*, through bacterial effector protein AvrA, increases β-catenin signaling (50). No evidence regarding *Fusobacterium nucleatum*-driven activation of β-catenin in melanoma was found. However, it can be speculated if *Fusobacterium nucleatum*, through FadA (a bacterial adhesin molecule), could bind to E-cadherin (which is present in melanocytes surface) (51). This interaction is crucial for bacterial internalization. Moreover, FadA also modulates E-cadherin, inhibiting its tumor suppression role and promoting the β-catenin pathway, which results in the upregulation of TCF/LEF transcription factors (such as MITF), cyclin D1, Myc, and NF-κB (whose role in melanomagenesis has been established) (25,26).

The **MAPK** pathway is a crucial signaling pathway implicated in cell proliferation and survival. Constitutive MAPK signaling may promote melanomagenesis through enhanced aberrant proliferation and survival, angiogenesis, and invasiveness. Specifically, the MAPK/ERK pathway is crucial in melanoma development (27,49).

*Mycobacterium leprae* has been proposed to induce ERK1/2 sustained phosphorylation, a mechanism that could be associated with tumorigenesis, including melanomagenesis, although this phenomenon was demonstrated in Schwann cells (52). Activation of pro-survival kinase ERK1/2 was also observed in neutrophils upon *Coxiella burnetii* infection (53).

Intracellular *Staphylococcus aureus* has been reported to deregulate the central carbon and amino acid metabolism and upregulate the ERK pathway as a response (54). Similarly, evidence supports that *Chlamydia trachomatis* infection activates MAPK/ERK signaling. In fact, in both *Chlamydia trachomatis* and *Staphylococcus aureus* was demonstrated that intracellular bacteria-triggered production of ROS could contribute to ERK stimulation (22,42,55). Specifically, following *Chlamydia trachomatis*-induced DNA damage, senescence-associated

heterochromatin foci (SAHF), which might lead to persistent cell proliferation, is upregulated in an ERK-dependent manner (42).

Activation of the **PI3K/AKT** pathway and subsequent phosphorylation of several targets to promote cell survival, proliferation, angiogenesis, invasiveness, and metabolic reprogramming has been reported in melanoma (27,49). *Coxiella burnetii*, *Mycobacterium tuberculosis*, and *Shigella* (through type III secretion effector IpgD) activate the PI3K/AKT pathway (5,13,53).

Through cagA (14), *Helicobacter pylori* may promote **SHP2** activation, a pro-oncogenic tyrosine phosphatase protein encoded by *PTPN11*, which is crucial in MAPK/ERK and PI3K/AKT signaling modulation. In this context, *PTPN11* expression is reportedly elevated in melanoma samples; consequently, SHP2 may contribute to melanoma development (56).

The **NF-κB** pathway is involved in the regulation of genes that promote cell proliferation, inhibition of apoptosis, invasiveness, and metastasis, and modulate immune and inflammatory responses. Deregulation of NF-κB at different levels has been described in malignant melanoma; in fact, several pathways that are altered in melanoma could influence NF-κB signaling (for instance, the PI3K/AKT pathway) (49).

Obligate intracellular bacteria *Chlamydia pneumoniae*, *Coxiella burnetii*, or *Rickettsia rickettsii* and facultative intracellular bacteria *Fusobacterium nucleatum* and *Neisseria gonorrhoeae* promote sustained transcription factor NF-κB activation dependent on intracellular and viable bacteria (13,57,58). Similarly, *Shigella* activates the NF-κB signaling pathway to counteract the oxidative stress triggered upon infection (5). Moreover, following internalization in the host cell through phagocytosis, *Legionella pneumophila* is able to assemble the Dot/Icm IVB secretion system to deliver protein substrates into the cytoplasm; a Dot/Icm-dependent upregulation of NF-κB has been described, thus leading to an anti-apoptotic effect (59).

Furthermore, it has been proposed that intracellular *Propionibacterium acnes* may also contribute to NF-κB activation (since NF-κB activation was higher in *Propionibacterium acnes*-invaded cells) through NOD1 and NOD2 interaction (NOD1 and NOD2 are members of the intracellular NOD-like receptor family) (60).

The correlation between NF-κB modulation and intracellular *Burkholderia cepacia* and *Francisella tularensis* is unclear. On the one hand, a study reported that NF-κB was upregulated upon *Burkholderia cepacia* infection, but it is undetermined whether this effect is specifically promoted by intracellular bacteria (48). On the other hand, intracellular toll-like receptors (TLR) could recognize intraphagosomal

*Francisella tularensis* and induce NF-κB signaling activation; however, this effect could also be promoted extracellularly through cell membrane receptors (61).

Through classical NF-κB activation, *Helicobacter pylori* and *Campylobacter jejuni* could trigger the expression of ubiquitin-editing enzyme A20 and suppress host cell apoptosis (62,63). Recently, the expression of **A20** has been found upregulated in melanoma cell lines, contributing to tumor growth, progression, invasion, and metastasis (64). Although this mechanism could establish a link between intracellular bacteria and melanomagenesis, bacterial internalization has not been assessed.

**HIF-1α** is also involved in different oncogenic signaling pathways (including the MAPK/ERK, PI3K/AKT/mTOR, JAK/STAT, NF-κB, Notch, and Wnt/β-catenin pathways) and metabolic reprogramming, both related to melanomagenesis. Moreover, HIF-1α is implicated in VEGF production (a mediator of angiogenesis, a hallmark of cancer cells) (27,65).

Intracellular replication of *Bartonella henselae* may be responsible for increased oxygen consumption and cellular hypoxia. The production of HIF-1 (which regulates the expression of VEGF genes) triggered by *Bartonella henselae* may be crucial in VEGF secretion (38). On the one hand, *Bartonella henselae* induces the secretion of pro-angiogenic factors (**VEGF** and **interleukin 8**) by endothelial cells. On the other hand, *Bartonella henselae* promotes the secretion of chemoattractants for monocytes/macrophages and polymorphonuclear cells, while also inducing VEGF secretion from these cells (38)*.* As described above, *Bartonella* species infection within melanoma cells is associated with increased pro-angiogenic cytokine expression that might influence melanoma development (66).

Inhibition of apoptosis is a common outcome following deregulation of several signaling pathways. In addition to the mechanisms described above, intracellular bacteria might be able to interfere with other **pro-survival** and **pro-apoptosis genes** or **proteins**.

For instance, upon *Coxiella burnetii* infection, pro-survival (*A1/Bfl-1* and *Bag1*) and pro-apoptosis genes (*Bax*, *Bim*, *CASP-2*, and *CASP-6*) expression increase and decrease, respectively; additionally, *Coxiella*-mediated regulation of Bcl-2 has been reported (13,67). In correlation with melanoma, pro-survival proteins (Bfl-1, Bag1, and Bcl-2) are found highly expressed in melanoma samples (68,69), while expression of pro-apoptosis molecules (Bax, Bim) is decreased (70). In contrast to caspase 2, caspase-6 activation may be involved in melanoma progression (71); thus, its suppression could inhibit its pro-tumoral effect.

Similarly, *Neisseria gonorrhoeae* inhibits apoptosis through upregulation of anti-apoptosis genes (*Bfl-1*, *c-IAP2*, *COX-2*, and *Mcl-1*) (72), ERK dephosphorylation, and NF-κB activation (as stated above) (57). Bfl-1 and COX-2 are highly expressed in melanoma cells and are involved in melanoma development (68,73). Mcl-1 also shows an increased expression in melanoma cells and is implicated in apoptosis inhibition and malignant cell proliferation promotion; however, Mcl-1 is regulated through the oncogenic activation of *BRAF* (74). Regarding ERK, although *Neisseria gonorrhoeae* induces its dephosphorylation (suppressing the ERK signaling pathway, involved in melanoma development), ERK inactivation correlates with a higher melanogenesis activity, which may play a role in melanomagenesis.

During its intracellular lifestyle, *Staphylococcus aureus*, through Type Seven Secretion System effector EsxA, may modulate cell death pathways and, consequently, inhibit apoptosis (23).

### 3.4.4.3    Inflammation

Deregulation of **inflammation** signaling pathways (and associated **inflammatory factors**) plays an active role in melanoma development.

Constitutive activation of the inflammasome, a cytoplasmic multimeric protein complex, induces enzymatic activation of canonical caspase-1 and subsequent secretion of pro-inflammatory cytokines (such as **IL-1β**) that may contribute to melanomagenesis (27). For instance, a study assessing host cell responses reported that intracellular *Fusobacterium nucleatum* infection led to NLRP3 inflammasome activation and NF-κB translocation to the nucleus, both increasing IL-1β secretion (25,58). This NF-κB-mediated response might include a pro-inflammatory gene signature, which could be associated with apoptosis inhibition, melanoma growth, and angiogenesis (27).

Cyclooxygenase-2 (**COX-2**), and subsequent prostaglandin E2 (PGE2) synthesis, are frequently overexpressed in several tumors (including melanoma); its role in carcinogenesis has been described. Currently, COX-2 upregulation associates with DNA damage, increased cell proliferation, evasion of apoptosis, invasiveness, metastasis, and a pro-inflammatory environment (73). A study demonstrated that the COX-2/PGE2 pathway was activated upon *Propionibacterium acnes* infection; the authors also reported that bacteria were located within intracellular vacuoles (41). Although it could be hypothesized that intracellular *Burkholderia cepacia* might upregulate COX-2 expression, a causal association between this outcome and intracellular localization of the bacteria has not been demonstrated (48).

Additionally, viable *Escherichia coli* within human macrophages reportedly induces COX-2 expression through the MAPK signaling pathway (19). Although this phenomenon has been reported in non-melanocyte/melanoma cells, melanoma-related macrophages may be involved in the regulation of the tumor microenvironment and could contribute to several stages of melanomagenesis (75). For instance, macrophages infected with *Mycobacterium tuberculosis* and *Salmonella typhimurium* release **exosomes** that may stimulate the immune and inflammatory response (76).

### 3.4.4.4 Immune response evasion

A study comparing the transcriptional response of dendritic cells against live intracellular *Salmonella enterica* demonstrated that intracellular *Salmonella* downregulated the antigen presentation sequence (77).

Melanocytes play a crucial role in the skin's immune response and, among its functions, are considered non-professional antigen presentation cells (78). As described above, a study reported the presentation of intracellular bacteria-derived peptides through HLA molecules in melanoma cells (36). Based on this evidence, it can be speculated whether intracellular bacteria may contribute to melanoma development via immunosilencing (a hallmark of cancer cells).

### 3.4.4.5 Metastasis

Deregulation of signal pathways that promote **metastasis** may also influence melanomagenesis.

As noted above, several pathways are implicated in cell migration and metastasis modulation; for instance, c-Myc and STAT-3 upregulate the expression of **EMT** genes, which enhance invasiveness and metastasis. For instance, since upregulation of EMT master regulator genes is observed during infection, *Mycobacterium leprae* might activate an EMT-like process that induces a mesenchymal stem-like phenotype, increasing its metastasic activity (79). Similarly, EMT-associated genes are upregulated upon *Chlamydia trachomatis* infection; thereby, these intracellular bacteria could trigger the malignant transformation of cells and promote metastasis (55).

In addition to EMT, melanoma-derived **exosomes** could reprogram the metabolism of stromal fibroblasts and promote aerobic glycolysis, thus favoring the creation of a pre-metastatic microenvironment. In this context, miR-155 is a microRNA whose presence within melanoma-derived exosomes may be crucial to its function (80). *Mycobacterium tuberculosis* and *Salmonella typhimurium* infection lead to exosome production and miR-155 upregulation (81); it could be

hypothesized whether exosomal microRNAs in melanoma are triggered by intracellular bacteria.

### 3.4.4.6    Epigenetic modifications

**Epigenetic modifications** (changes in gene expression that do not involve an alteration of the DNA sequence) may play a crucial role in melanoma. Intracellular bacteria reportedly induce epigenetic changes and influence melanoma development.

For instance, upon *Mycobacterium tuberculosis* infection, secretion of lipoprotein LpqH, which may be secreted from viable intracellular mycobacteria (82), induces CCAAT/enhancer-binding protein beta activation (CEBPβ) and loss of function of SWItch/Sucrose NonFermentable (SWI/SNF, a chromatin remodeling complex with a tumor suppressor role). Both changes inactivate gene encoding the major histocompatibility complex (MHC) class II transactivator and could trigger immune evasion; moreover, CEBPβ has been reported to induce metastasis through MITF suppression (76,83).

*Legionella* and *Burkholderia thailandensis* may also contribute to melanomagenesis through epigenetic changes since both promote H3K4 methylation, a key alteration associated with malignant melanoma (27,84,85).

Modification of non-coding RNA may be involved in metabolic reprogramming and the establishment of a pre-metastatic niche in melanoma (27). As discussed above, some intracellular bacteria might be able to modulate their expression and induce oncogenesis.

### 3.4.4.7    Deregulation of cellular energetics

Several intracellular bacteria may play a role in tumorigenesis through **bacteria-induced host cell metabolic reprogramming** since metabolism deregulation is a recognized hallmark in cancer cells.

The host cell metabolism can not meet the enormous biosynthetic requirements for successful survival of intracellular bacteria. Several intracellular bacteria rely on their ability to reprogram the host cell metabolism to a Warburg-like state; that is, a metabolic bioenergetic shift to aerobic glycolysis (instead of oxidative phosphorylation), increasing cellular glucose uptake and lactate production. Subsequently, glycolysis and tricarboxylic acid cycle intermediates are redirected toward the synthesis of energy sources (fatty acids, lipids, amino acids, and nucleotides). Moreover, lipid metabolism alteration by intracellular bacteria has also been reported (86).

Several mechanisms involved in metabolic reprogramming, which are intimately bound to tumorigenesis (87), could be induced by intracellular bacteria to ensure its survival. Altered HIF-1, p53, and PI3K/mTOR signaling are the major responsible for bacterial-driven host cell metabolism deregulation; as described above, these changes may also play a role in melanomagenesis.

*Bartonella henselae*, *Chlamydia trachomatis*, *Mycobacterium tuberculosis*, and *Salmonella enterica* increase **HIF-1** activation (87). HIF-1 upregulates the expression of genes encoding glucose transporters and enzymes involved in the glycolytic and pentose-phosphate pathways, leading to increased glucose uptake and aerobic glycolysis (65).

*Brucella abortus*, *Chlamydia trachomatis*, *Coxiella burnetii*, *Francisella tularensis*, *Legionella pneumophila*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Salmonella enterica*, and *Shigella flexneri* may activate the **PI3K/AKT pathway**. *Brucella abortus, Francisella tularensis*, *Legionella pneumophila*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Salmonella enterica*, and *Shigella flexneri* are also involved in **mTOR** activation (87).

Activation of the PI3K/AKT pathway and downstream mTOR complex 1 deregulate cell metabolism. Evidence supports its role in promoting membrane localization of glucose transporter 1 (GLUT 1), glucose uptake, and activation of enzymes involved in glycolysis and the pentose phosphate pathways while also increasing lipid and protein biosynthesis (88).

**p53** is implicated in the modulation of the Warburg effect, lipid and amino acid metabolism, and the regulation of other pathways. Intracellular bacteria, such as *Mycobacterium tuberculosis* and *Salmonella enterica*, upregulate p53 expression, leading to suppression of the Warburg effect and the PI3K/AKT/mTOR pathway (with a similar outcome). Alternatively, *Chlamydia trachomatis* and *Listeria monocytogenes* downregulate p53 expression, hence stimulating aerobic glycolysis, in addition to the loss of its tumor-suppressor function (which could contribute to tumor development) (87).

**Myc** and **MITF** activation by *Chlamydia trachomatis* and *Legionella pneumophila*, respectively, may be involved in host cell metabolism rewiring (including aerobic glycolysis, lipid, and amino acid biosynthesis), thus inducing cancer cell growth and proliferation (27).

### 3.4.4.8   Lateral gene transfer

**Lateral gene transfer** from intracellular bacteria into eukaryotic host genomes has been described. A study reported that attenuated intracellular bacteria (invasive *Escherichia coli*, *Listeria*, *Salmonella*, and *Shigella*) could deliver

bacterial DNA in several mammalian (phagocytic and non-phagocytic) cell types (89). Another study demonstrated DNA transfer by intracellular *Coxiella burnetii* and *Legionella pneumophila* through the Dot/Icm Type IV secretion system (90).

It has been proposed that bacterial DNA integrations may constitute a mechanism for the disruption of gene function or acquisition and expression of novel genes, which could suggest a crucial role in carcinogenesis; in fact, evidence supports that detection of these integrations is more frequent in tumor than in non-tumor cells (91). However, the hypothesis that bacterial DNA integration into the human genome may induce tumorigenesis (specifically, melanomagenesis) has not been demonstrated (91).

### 3.4.4.9 Melanin-producing bacteria

Microbial production of melanin has been described; in fact, **microbial melanization** might be associated with virulence in several microorganisms (92).

Since melanogenesis may be involved in melanoma pathogenesis, we speculate whether microbial melanin synthesis within the cell might enhance melanomagenesis.

Predominantly, two pathways are responsible for microbial melanin synthesis: the 3,4-dihydroxyphenylalanine (DOPA) pathway (through the transformation of tyrosine by tyrosinase and laccase enzymes, similar to the mammalian melanin synthesis) or the 1,8-dihydroxynaphthalene (DHN) pathway (through malonyl-CoA transformation) (92).

Among melanin-producing bacteria, multiple human pathogens such as *Bordetella pertussis/parapertussis, Burkholderia cepacia*, *Escherichia coli*, *Legionella pneumophila*, or *Mycobacterium* species have been identified (such as *Mycobacterium leprae*) (93). For instance, melanin produced by *Bordetella parapertussis* is involved in its intracellular survival within macrophages (94).

Although these organisms are classified as intracellular bacteria (mainly facultative, except *Mycobacterium leprae*, an obligate intracellular bacteria), no evidence was found regarding melanin production inside the host cell, interference with melanogenesis in melanocytes, and melanomagenesis.

### 3.4.4.10 Melanogenesis interference

Melanocytes contribute to the skin's innate and adaptative immune response. Since melanocytes are capable of phagocytosis of pathogens, a crucial event in antigen processing and presentation, a role as non-professional antigen-presenting cells has been proposed (78). Reportedly, melanin displays

antimicrobial activity and can physically trap infectious microorganisms, that is, melanin may be crucial for melanocytes to achieve **immunocompetence** (95).

Human melanocytes have been shown to express TLR 1, 2, 3, 4, 6, 7, and 9. Following pathogen recognition, TLRs are involved in the innate immune response. However, some of them are able to promote (TLR2, TLR4, TLR9) or inhibit (TLR3, TLR5, TLR7) melanogenesis; particularly, TLR3, TLR7, and TLR9 are endosomal innate immune sensors and are located in endocytic vesicles (intracellular) (78,96). Although intracellular bacteria recognition by TLR might lead to a deregulation of melanogenesis and contribute to the process of melanomagenesis, no evidence was found.

Some bacteria, such as *Mycobacterium leprae*, have been reported to infect human melanocytes and deregulate metabolic functions, leading to hypopigmentation (97). Since melanin plays an active role in melanocyte immunocompetence, it can be speculated that downregulation of melanin production might induce immunosilencing. However, an association between *Mycobacterium leprae* and melanoma development has not been demonstrated.

As discussed above, several **signaling pathways** could be altered by intracellular bacteria. It could be hypothesized that, in addition to the previously described effect, intracellular bacteria-driven alteration of MITF, NF-κB, p53, and Wnt/β-catenin could modulate melanogenesis and, therefore, melanomagenesis. Activation of the MAPK/ERK and PI3K/AKT pathways could be an exception since some studies report that its upregulation (a common effect of bacteria) might decrease melanogenesis; nevertheless, it remains unknown how ERK and PI3K/AKT downregulation of melanogenesis would interfere with the rest of pro-melanomagenesis signaling (29).

Another mechanism that may be associated is **ROS** production. Its role in melanogenesis and melanomagenesis has been discussed previously. Evidence supports that several intracellular bacteria could induce high levels of intracellular ROS. For instance, intracellular *Chlamydia trachomatis*, *Staphylococcus aureus*, *Shigella*, and *Propionibacterium acnes* trigger oxidative stress and promote ROS production and its subsequent outcomes (5,22,41). However, there is no conclusive evidence regarding melanomagenesis.

Intracellular bacteria have been reported to hijack **mitochondrial dynamics**, which has been related to melanogenesis. For instance, *Legionella pneumophila*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Salmonella enterica*, and *Shigella flexneri* induce mitochondrial fission, while *Chlamydia trachomatis* promotes mitochondrial fusion (98). Although mitochondrial fusion and fission enhance and inhibit melanogenesis, respectively (and apparently, modulate

melanomagenesis), the correlation between bacteria-driven mitochondrial dynamics with melanoma development remains unclear.

# 4    Conclusions

Intracellular bacteria's contribution to tumorigenesis is increasingly apparent. Specifically, the evidence so far has only identified *Bartonella henselae*, *Fusobacterium nucleatum*, *Mycoplasma hyorhinis*, and *Staphylococcus aureus* within melanoma cells, and its role in melanomagenesis remains unclear. However, it should be taken into account that intracellular bacteria might modulate tumorigenesis through invasion of not only melanoma cells but tumor-infiltrating immune cells.

There are several hypothetical mechanisms (deregulation of signaling pathways, immunosilencing, metabolic reprogramming, lateral gene transfer, or even melanogenesis interference) through which intracellular bacteria could contribute to melanoma genesis, progression, and dissemination. However, these mechanisms have only been reported in other non-melanoma tumors; moreover, it has been suggested that the changes driven by facultative intracellular bacteria (such as *Fusobacterium nucleatum*) may be independent of its internalization.

A casual association between these bacteria and melanoma can not be established. However, given the higher abundance of some of these bacteria in malignant melanoma, and the promotion of mechanisms related to melanoma development, it can be hypothesized that these bacteria might influence this process at some degree.

The possibility of an intracellular bacteria-driven interference leading to melanomagenesis would allow a deeper understanding of the complexity of tumorigenesis and the development of different therapeutic approaches. Further studies are needed to obtain evidence that could support or rule out this hypothesis.

# 5    References

1.    Alonso A, García-del Portillo F. Hijacking of eukaryotic functions by intracellular bacterial pathogens. Int Microbiol. 2004 Sep;7(3):181–91.
2.    Schulz F, Horn M. Intranuclear bacteria: inside the cellular control center of eukaryotes. Trends Cell Biol. 2015 Jun;25(6):339–46.
3.    Haas A. The Phagosome: Compartment with a License to Kill. Traffic. 2007 Apr;8(4):311–30.
4.    Lebreton A, Stavru F, Cossart P. Organelle targeting during bacterial infection: insights from Listeria. Trends Cell Biol. 2015 Jun;25(6):330–8.
5.    Schnupf P, Sansonetti PJ. *Shigella* Pathogenesis: New Insights through Advanced Methodologies. Microbiol Spectr. 2019 Apr;7(2).

6. Livengood JA, Gilmore RD. Invasion of human neuronal and glial cells by an infectious strain of Borrelia burgdorferi. Microbes Infect. 2006 Nov;8(14–15):2832–40.

7. Mekadim C, Skalnikova HK, Cizkova J, Cizkova V, Palanova A, Horak V, et al. Dysbiosis of skin microbiome and gut microbiome in melanoma progression. BMC Microbiol. 2022 Dec;22(1):63.

8. Nejman D, Livyatan I, Fuks G, Gavert N, Zwang Y, Geller LT, et al. The human tumor microbiome is composed of tumor type–specific intracellular bacteria. Science (1979). 2020 May;368(6494):973–80.

9. Dong Q lin, Xing X ying. Cancer cells arise from bacteria. Cancer Cell Int. 2018 Dec 14;18(1):205.

10. Arcia Franchini AP, Iskander B, Anwer F, Oliveri F, Fotios K, Panday P, et al. The Role of Chlamydia Trachomatis in the Pathogenesis of Cervical Cancer. Cureus. 2022 Jan;14(1):e21331.

11. Wang C, Zhang N, Gao L. Association between Chlamydia pneumoniae infection and lung cancer: a meta-analysis. Transl Cancer Res. 2019 Dec;8(8):2813–9.

12. Melenotte C, Mezouar S, Ben Amara A, Benatti S, Chiaroni J, Devaux C, et al. A transcriptional signature associated with non-Hodgkin lymphoma in the blood of patients with Q fever. PLoS One. 2019 Jun;14(6):e0217542.

13. Hussain SK, Voth DE. Coxiella Subversion of Intracellular Host Signaling. In 2012. p. 131–40.

14. Dubois A. Intracellular Helicobacter pylori and Gastric Carcinogenesis: An "Old" Frontier Worth Revisiting. Gastroenterology. 2007 Mar;132(3):1177–80.

15. Tsugawa H, Mori H, Matsuzaki J, Sato A, Saito Y, Imoto M, et al. CAPZA1 determines the risk of gastric carcinogenesis by inhibiting *Helicobacter pylori* CagA-degraded autophagy. Autophagy. 2019 Feb;15(2):242–58.

16. Scanu T, Spaapen RM, Bakker JM, Pratap CB, Wu L en, Hofland I, et al. Salmonella Manipulation of Host Signaling Pathways Provokes Cellular Transformation Associated with Gallbladder Carcinoma. Cell Host Microbe. 2015 Jun;17(6):763–74.

17. Lu R, Zhang Y guo, Sun J. STAT3 activation in infection and infection-associated cancer. Mol Cell Endocrinol. 2017 Aug;451:80–7.

18. Wu H, Jones RM, Neish AS. The Salmonella effector AvrA mediates bacterial intracellular survival during infection in vivo. Cell Microbiol. 2012 Jan;14(1):28–39.

19. Raisch J, Rolhion N, Dubois A, Darfeuille-Michaud A, Bringer MA. Intracellular colon cancer-associated Escherichia coli promote protumoral activities of human macrophages by inducing sustained COX-2 expression. Laboratory Investigation. 2015 Mar;95(3):296–307.

20. Chai Q, Lu Z, Liu Z, Zhong Y, Zhang F, Qiu C, et al. Lung gene expression signatures suggest pathogenic links and molecular markers for pulmonary

tuberculosis, adenocarcinoma and sarcoidosis. Commun Biol. 2020 Oct;3(1):604.

21. Gupta PK, Tripathi D, Kulkarni S, Rajan MGR. Mycobacterium tuberculosis H37Rv infected THP-1 cells induce epithelial mesenchymal transition (EMT) in lung adenocarcinoma epithelial cell line (A549). Cell Immunol. 2016 Feb;300:33–40.

22. Deplanche M, Mouhali N, Nguyen MT, Cauty C, Ezan F, Diot A, et al. Staphylococcus aureus induces DNA damage in host cell. Sci Rep. 2019 May;9(1):7694.

23. Soe YM, Bedoui S, Stinear TP, Hachani A. Intracellular <scp> Staphylococcus aureus </scp> and host cell death pathways. Cell Microbiol. 2021 May 24;23(5):e13317.

24. Ou S, Wang H, Tao Y, Luo K, Ye J, Ran S, et al. Fusobacterium nucleatum and colorectal cancer: From phenomenon to mechanism. Front Cell Infect Microbiol. 2022 Nov;12:1020583.

25. Gholizadeh P, Eslami H, Kafil HS. Carcinogenesis mechanisms of Fusobacterium nucleatum. Biomedicine & Pharmacotherapy. 2017 May;89:918–25.

26. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum Promotes Colorectal Carcinogenesis by Modulating E-Cadherin/β-Catenin Signaling via its FadA Adhesin. Cell Host Microbe. 2013 Aug;14(2):195–206.

27. Guo W, Wang H, Li C. Signal pathways of melanoma and targeted therapy. Signal Transduct Target Ther. 2021 Dec;6(1):424.

28. Cannavò SP, Tonacci A, Bertino L, Casciaro M, Borgia F, Gangemi S. The role of oxidative stress in the biology of melanoma: A systematic review. Pathol Res Pract. 2019 Jan;215(1):21–8.

29. Su TR, Lin JJ, Tsai CC, Huang TK, Yang ZY, Wu MO, et al. Inhibition of Melanogenesis by Gallic Acid: Possible Involvement of the PI3K/Akt, MEK/ERK and Wnt/β-Catenin Signaling Pathways in B16F10 Cells. Int J Mol Sci. 2013 Oct;14(10):20443–58.

30. Lee SE, Park SH, Oh SW, Yoo JA, Kwon K, Park SJ, et al. Beauvericin inhibits melanogenesis by regulating cAMP/PKA/CREB and LXR-α/p38 MAPK–mediated pathways. Sci Rep. 2018 Oct;8(1):14958.

31. Kamiński K, Kazimierczak U, Kolenda T. Oxidative stress in melanogenesis and melanoma development. Współczesna Onkologia. 2022;26(1):1–7.

32. Kim ES, Park SJ, Goh MJ, Na YJ, Jo DS, Jo YK, et al. Mitochondrial dynamics regulate melanogenesis through proteasomal degradation of MITF via ROS-ERK activation. Pigment Cell Melanoma Res. 2014 Nov;27(6):1051–62.

33. Kazimierczak U, Dondajewska E, Zajaczkowska M, Karwacka M, Kolenda T, Mackiewicz A. LATS1 Is a Mediator of Melanogenesis in Response to Oxidative Stress and Regulator of Melanoma Growth. Int J Mol Sci. 2021 Mar;22(6):3108.

34. Zhang X, Li H, Liu C, Yuan X. Role of ROS-mediated autophagy in melanoma (Review). Mol Med Rep. 2022 Aug;26(4):303.

35. Slominski RM, Sarna T, Płonka PM, Raman C, Brożyna AA, Slominski AT. Melanoma, Melanin, and Melanogenesis: The Yin and Yang Relationship. Front Oncol. 2022 Mar;12:842496.

36. Kalaora S, Nagler A, Nejman D, Alon M, Barbolin C, Barnea E, et al. Identification of bacteria-derived HLA-bound peptides in melanoma. Nature. 2021 Apr;592(7852):138–43.

37. Ericson ME, Breitschwerdt EB, Reicherter P, Maxwell C, Maggi RG, Melvin RG, et al. Bartonella henselae Detected in Malignant Melanoma, a Preliminary Study. Pathogens. 2021 Mar;10(3):326.

38. Pulliainen AT, Dehio C. Persistence of *Bartonella* spp. stealth pathogens: from subclinical infections to vasoproliferative tumor formation. FEMS Microbiol Rev. 2012 May;36(3):563–99.

39. Kornspan JD, Tarshis M, Rottem S. Invasion of Melanoma Cells by *Mycoplasma hyorhinis*: Enhancement by Protease Treatment. Infect Immun. 2010 Feb;78(2):611–7.

40. Gedye C, Cardwell T, Dimopoulos N, Tan BS, Jackson H, Svobodová S, et al. Mycoplasma Infection Alters Cancer Stem Cell Properties in Vitro. Stem Cell Rev Rep. 2016 Feb;12(1):156–61.

41. Fassi Fehri L, Mak TN, Laube B, Brinkmann V, Ogilvie LA, Mollenkopf H, et al. Prevalence of Propionibacterium acnes in diseased prostates and its inflammatory and transforming activity on prostate epithelial cells. International Journal of Medical Microbiology. 2011 Jan;301(1):69–78.

42. Chumduri C, Gurumurthy RK, Zadora PK, Mi Y, Meyer TF. Chlamydia Infection Promotes Host DNA Damage and Proliferation but Impairs the DNA Damage Response. Cell Host Microbe. 2013 Jun;13(6):746–58.

43. Bhandaru M, Martinka M, McElwee KJ, Rotte A. Prognostic Significance of Nuclear Phospho-ATM Expression in Melanoma. PLoS One. 2015 Aug;10(8):e0134678.

44. Lin X, Sun R, Zhao X, Zhu D, Zhao X, Gu Q, et al. C-myc overexpression drives melanoma metastasis by promoting vasculogenic mimicry via c-myc/snail/Bax signaling. J Mol Med. 2017 Jan 19;95(1):53–67.

45. Sun J, Hobert ME, Rao AS, Neish AS, Madara JL. Bacterial activation of β-catenin signaling in human epithelia. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2004 Jul;287(1):G220–7.

46. Alekseenko A, Wojas-Pelc A, Lis GJ, Wiśniowski Z, Czerwińska M, Niewiara Ł, et al. Expression of cyclins A and E in melanocytic skin lesions and its correlation with some clinicopathologic features. Folia Histochem Cytobiol. 2012 Jul;50(2):263–9.

47. Loureiro JB, Raimundo L, Calheiros J, Carvalho C, Barcherini V, Lima NR, et al. Targeting p53 for Melanoma Treatment: Counteracting Tumour Proliferation, Dissemination and Therapeutic Resistance. Cancers (Basel). 2021 Apr;13(7):1648.

48. Mariappan V, Vellasamy KM, Thimma J, Hashim OH, Vadivelu J. Infection of Burkholderia cepacia Induces Homeostatic Responses in the Host for Their Prolonged Survival: The Microarray Perspective. PLoS One. 2013 Oct;8(10):e77418.

49. Dantonio PM, Klein MO, Freire MRVB, Araujo CN, Chiacetti AC, Correa RG. Exploring major signaling cascades in melanomagenesis: a rationale route for targetted skin cancer therapy. Biosci Rep. 2018 Oct;38(5):BSR20180511.

50. Lu R, Wu S, Zhang Y g, Xia Y, Liu X, Zheng Y, et al. Enteric bacterial protein AvrA promotes colonic tumorigenesis and activates colonic beta-catenin signaling pathway. Oncogenesis. 2014 Jun;3(6):e105–e105.

51. Tang A, Eller MS, Hara M, Yaar M, Hirohashi S, Gilchrest BA. E-cadherin is the major mediator of human melanocyte adhesion to keratinocytes in vitro. J Cell Sci. 1994 Apr;107(4):983–92.

52. Voth DE, Heinzen RA. Sustained Activation of Akt and Erk1/2 Is Required for *Coxiella burnetii* Antiapoptotic Activity. Infect Immun. 2009 Jan;77(1):205–13.

53. Cherla R, Zhang Y, Ledbetter L, Zhang G. Coxiella burnetii Inhibits Neutrophil Apoptosis by Exploiting Survival Pathways and Antiapoptotic Protein Mcl-1. Infect Immun. 2018 Apr;86(4):e00504-17.

54. Bravo-Santano N, Ellis JK, Mateos LM, Calle Y, Keun HC, Behrends V, et al. Intracellular Staphylococcus aureus Modulates Host Central Carbon Metabolism To Activate Autophagy. mSphere. 2018 Aug;3(4):e00374-18.

55. Zadora PK, Chumduri C, Imami K, Berger H, Mi Y, Selbach M, et al. Integrated Phosphoproteome and Transcriptome Analysis Reveals Chlamydia-Induced Epithelial-to-Mesenchymal Transition in Host Cells. Cell Rep. 2019 Jan;26(5):1286-1302.e8.

56. Zhang RY, Yu ZH, Zeng L, Zhang S, Bai Y, Miao J, et al. SHP2 phosphatase as a novel therapeutic target for melanoma treatment. Oncotarget. 2016 Nov;7(45):73817–29.

57. Liu GL, Parti RP, Dillon JAR. Suppression of ERK activation in urethral epithelial cells infected with Neisseria gonorrhoeae and its isogenic minD mutant contributes to anti-apoptosis. Microbes Infect. 2015 Apr;17(4):317–22.

58. Bui FQ, Johnson L, Roberts J, Hung SC, Lee J, Atanasova KR, et al. *Fusobacterium nucleatum* infection of gingival epithelial cells leads to NLRP3 inflammasome-dependent secretion of IL-1β and the danger signals ASC and HMGB1. Cell Microbiol. 2016 Jul;18(7):970–81.

59. Losick VP, Isberg RR. NF-κB translocation prevents host cell death after low-dose challenge by Legionella pneumophila. Journal of Experimental Medicine. 2006 Sep;203(9):2177–89.

60. Bae Y, Ito T, Iida T, Uchida K, Sekine M, Nakajima Y, et al. Intracellular Propionibacterium acnes Infection in Glandular Epithelium and Stromal

Macrophages of the Prostate with or without Cancer. PLoS One. 2014 Feb 28;9(2):e90324.

61. Kubelkova K, Macela A. Innate Immune Recognition: An Issue More Complex Than Expected. Front Cell Infect Microbiol. 2019 Jul;9:241.

62. Lim MCC, Maubach G, Birkl-Toeglhofer AM, Haybaeck J, Vieth M, Naumann M. A20 undermines alternative NF-κB activity and expression of anti-apoptotic genes in Helicobacter pylori infection. Cellular and Molecular Life Sciences. 2022 Feb;79(2):102.

63. Lim MCC, Maubach G, Sokolova O, Feige MH, Diezko R, Buchbinder J, et al. Pathogen-induced ubiquitin-editing enzyme A20 bifunctionally shuts off NF-κB and caspase-8-dependent apoptotic cell death. Cell Death Differ. 2017 Sep;24(9):1621–31.

64. Ma J, Wang H, Guo S, Yi X, Zhao T, Liu Y, et al. A20 promotes melanoma progression via the activation of Akt pathway. Cell Death Dis. 2020 Sep;11(9):794.

65. Malekan M, Ebrahimzadeh MA, Sheida F. The role of Hypoxia-Inducible Factor-1alpha and its signaling in melanoma. Biomedicine & Pharmacotherapy. 2021 Sep;141:111873.

66. Ericson ME, Breitschwerdt EB, Reicherter P, Maxwell C, Maggi RG, Melvin RG, et al. Bartonella henselae Detected in Malignant Melanoma, a Preliminary Study. Pathogens. 2021 Mar;10(3):326.

67. Cordsmeier A, Rinkel S, Jeninga M, Schulze-Luehrmann J, Ölke M, Schmid B, et al. The Coxiella burnetii T4SS effector protein AnkG hijacks the 7SK small nuclear ribonucleoprotein complex for reprogramming host cell transcription. PLoS Pathog. 2022 Feb;18(2):e1010266.

68. Hind CK, Carter MJ, Harris CL, Chan HTC, James S, Cragg MS. Role of the pro-survival molecule Bfl-1 in melanoma. Int J Biochem Cell Biol. 2015 Feb;59:94–102.

69. D'Arcangelo D, Giampietri C, Muscio M, Scatozza F, Facchiano F, Facchiano A. WIPI1, BAG1, and PEX3 Autophagy-Related Genes Are Relevant Melanoma Markers. Oxid Med Cell Longev. 2018 Dec;2018:1–12.

70. Sheridan C, Brumatti G, Martin SJ. Oncogenic B-RafV600E Inhibits Apoptosis and Promotes ERK-dependent Inactivation of Bad and Bim. Journal of Biological Chemistry. 2008 Aug;283(32):22128–35.

71. Woenckhaus C, Giebel J, Failing K, Fenic I, Dittberner T, Poetsch M. Expression of AP-2?, c-kit, and cleaved caspase-6 and -3 in naevi and malignant melanomas of the skin. A possible role for caspases in melanoma progression? J Pathol. 2003 Oct;201(2):278–87.

72. Binnicker MJ, Williams RD, Apicella MA. Infection of human urethral epithelium with Neisseria gonorrhoeae elicits an upregulation of host anti-apoptotic factors and protects cells from staurosporine-induced apoptosis. Cell Microbiol. 2003 Aug;5(8):549–60.

73. Valentina Tudor D, Bâldea I, Lupu M, Kacso T, Kutasi E, Hopârtean A, et al. COX-2 as a potential biomarker and therapeutic target in melanoma. Cancer Biol Med. 2020;17(1):20–31.

74. Wang H, Guo M, Wei H, Chen Y. Targeting MCL-1 in cancer: current status and perspectives. J Hematol Oncol. 2021 Dec;14(1):67.

75. Pieniazek M, Matkowski R, Donizy P. Macrophages in skin melanoma-the key element in melanomagenesis (Review). Oncol Lett. 2018 Feb;15(4):5399–404.

76. Silmon de Monerri NC, Kim K. Pathogens Hijack the Epigenome. Am J Pathol. 2014 Apr;184(4):897–911.

77. Aulicino A, Rue-Albrecht KC, Preciado-Llanes L, Napolitani G, Ashley N, Cribbs A, et al. Invasive Salmonella exploits divergent immune evasion strategies in infected and bystander dendritic cell subsets. Nat Commun. 2018 Nov;9(1):4883.

78. Chen YY, Liu LP, Zhou H, Zheng YW, Li YM. Recognition of Melanocytes in Immuno-Neuroendocrinology and Circadian Rhythms: Beyond the Conventional Melanin Synthesis. Cells. 2022 Jun;11(13):2082.

79. Hess S, Rambukkana A. Bacterial-induced cell reprogramming to stem cell-like cells: new premise in host–pathogen interactions. Curr Opin Microbiol. 2015 Feb;23:179–88.

80. Shu S La, Yang Y, Allen CL, Maguire O, Minderman H, Sen A, et al. Metabolic reprogramming of stromal fibroblasts by melanoma exosome microRNA favours a pre-metastatic microenvironment. Sci Rep. 2018 Aug;8(1):12905.

81. Ahmed W, Zheng K, Liu ZF. Small Non-Coding RNAs: New Insights in Modulation of Host Immune Response by Intracellular Bacterial Pathogens. Front Immunol. 2016 Oct;7:431.

82. Harding C v., Boom WH. Regulation of antigen presentation by Mycobacterium tuberculosis: a role for Toll-like receptors. Nat Rev Microbiol. 2010 Apr;8(4):296–307.

83. Swoboda A, Soukup R, Eckel O, Kinslechner K, Wingelhofer B, Schörghofer D, et al. STAT3 promotes melanoma metastasis by CEBP-induced repression of the MITF pathway. Oncogene. 2021 Feb;40(6):1091–105.

84. Dong W, Hamon MA. Revealing eukaryotic histone-modifying mechanisms through bacterial infection. Semin Immunopathol. 2020 Apr;42(2):201–13.

85. Raman AT, Rai K. Loss of histone acetylation and H3K4 methylation promotes melanocytic malignant transformation. Mol Cell Oncol. 2018 May;5(3):e1359229.

86. Proal AD, VanElzakker MB. Pathogens Hijack Host Cell Metabolism: Intracellular Infection as a Driver of the Warburg Effect in Cancer and Other Chronic Inflammatory Conditions. Immunometabolism. 2021 Jan;3(1):e210003.

87. Eisenreich W, Rudel T, Heesemann J, Goebel W. How Viral and Intracellular Bacterial Pathogens Reprogram the Metabolism of Host Cells

to Allow Their Intracellular Replication. Front Cell Infect Microbiol. 2019 Mar;9:42.

88. Hoxhaj G, Manning BD. The PI3K–AKT network at the interface of oncogenic signalling and cancer metabolism. Nat Rev Cancer. 2020 Feb;20(2):74–88.

89. Grillot-Courvalin C. Bacteria as gene delivery vectors for mammalian cells Commentary. Curr Opin Biotechnol. 1999 Oct;10(5):477–81.

90. Guzmán-Herrador DL, Steiner S, Alperi A, González-Prieto C, Roy CR, Llosa M. DNA Delivery and Genomic Integration into Mammalian Target Cells through Type IV A and B Secretion Systems of Human Pathogens. Front Microbiol. 2017 Aug;8:1503.

91. Riley DR, Sieber KB, Robinson KM, White JR, Ganesan A, Nourbakhsh S, et al. Bacteria-Human Somatic Cell Lateral Gene Transfer Is Enriched in Cancer Samples. PLoS Comput Biol. 2013 Jun;9(6):e1003107.

92. Tran-Ly AN, Reyes C, Schwarze FWMR, Ribera J. Microbial production of melanin and its various applications. World J Microbiol Biotechnol. 2020 Nov;36(11):170.

93. Urán ME, Cano LE. Melanina: Implicaciones en la patogénesis de algunas enfermedades y su capacidad de evadir la respuesta inmune del hospedero. Infectio. 2008;12(2):128–48.

94. Hiramatsu Y, Nishida T, Nugraha DK, Sugihara F, Horiguchi Y. Melanin Produced by *Bordetella parapertussis* Confers a Survival Advantage to the Bacterium during Host Infection. mSphere. 2021 Oct;6(5):e00819-21.

95. Mackintosh JA. The Antimicrobial Properties of Melanocytes, Melanosomes and Melanin and the Evolution of Black Skin. J Theor Biol. 2001 Jul;211(2):101–13.

96. Koike S, Yamasaki K. Melanogenesis Connection with Innate Immunity and Toll-Like Receptors. Int J Mol Sci. 2020 Dec;21(24):9769.

97. Gasque P, Jaffar-Bandjee MC. The immunology and inflammatory responses of human melanocytes in infectious diseases. Journal of Infection. 2015 Oct;71(4):413–21.

98. Spier A, Stavru F, Cossart P. Interaction between Intracellular Bacterial Pathogens and Host Cell Mitochondria. Microbiol Spectr. 2019 Apr;7(2).