

Review article:

**MYCOPLASMAS AND CANCER:
FOCUS ON NUCLEOSIDE METABOLISM**

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ABSTRACT

The standard of care for patients suffering cancer often includes treatment with nucleoside analogues (NAs). NAs are internalized by cell-specific nucleobase/nucleoside transporters and, after enzymatic activation (often one or more phosphorylation steps), interfere with cellular nucleo(s)tide metabolism and DNA/RNA synthesis. Therefore, their efficacy is highly dependent on the expression and activity of nucleo(s)tide-metabolizing enzymes, and alterations thereof (e.g. by down/upregulated expression or mutations) may change the susceptibility to NA-based therapy and/or confer drug resistance. Apart from host cell factors, several other variables including microbial presence may determine the metabolome (i.e. metabolite concentrations) of human tissues. Studying the diversity of microorganisms that are associated with the human body has already provided new insights in several diseases (e.g. diabetes and inflammatory bowel disease) and the metabolic exchange between tissues and their specific microbiota was found to affect the bioavailability and toxicity of certain anticancer drugs, including NAs. Several studies report a preferential colonization of tumor tissues with some mycoplasma species (mostly *Mycoplasma hyorhinitis*). These prokaryotes are also a common source of cell culture contamination and alter the cytostatic activity of some NAs *in vitro* due to the expression of nucleoside-catabolizing enzymes. Mycoplasma infection may therefore bias experimental work with NAs, and their presence in the tumor microenvironment could be of significance when optimizing nucleoside-based cancer treatment.

Keywords: cancer, nucleoside analogues, chemotherapy, mycoplasma, nucleoside phosphorylase, *Mycoplasma hyorhinitis*

**NUCLEOSIDE METABOLISM
AND THERAPEUTIC
ANTIMETABOLITES**

Biosynthesis of nucleo(s)tides

Pyrimidine and purine nucleotides are crucial in various cellular processes. They represent the building blocks for the synthesis of nucleic acids (DNA and RNA), provide cellular energy [e.g. adenosine-5'-triphosphate (ATP)], are involved as conjugates in biosynthetic processes (e.g. uri-

dine-5'-diphosphate-glucose), produce signaling molecules (e.g. cyclic adenosine-5'-monophosphate and cyclic guanosine-5'-monophosphate) or act as enzyme cofactors (e.g. nicotinamide adenine dinucleotide, flavin adenine dinucleotide, coenzyme A). The cellular requirements for nucleotides can be met either by (i) *de novo* synthesis starting from low molecular weight precursors or (ii) the salvage pathway, recycling nucleosides or nucleobases

from sources within the body or from food (Hatse et al., 1999).

Pyrimidine nucleotide synthesis/ metabolism

De novo synthesis of pyrimidine nucleotides

As visualized in Figure 1, pyrimidine nucleotides can be assembled by the *de novo* synthetic pathway from L-glutamine, L-aspartic acid and 5-phosphoribosyl-1-pyrophosphate (PRPP) in a 6-step reaction process resulting in uridine-5'-monophosphate (UMP) which is a precursor for other pyrimidine nucleotides. Nucleotide kinase activity converts UMP into uridine-5'-diphosphate (UDP) and uridine-5'-triphosphate (UTP). Amination of UTP by the rate-limiting CTP synthetase produces cytidine-5'-triphosphate (CTP) and both CTP and UTP are incorporated into RNA. The formation of 2'-deoxyribonucleotides from UDP and cytidine-5'-diphosphate (CDP) is catalyzed by ribonucleotide reductase (RR) resulting in the production of 2'-deoxyuridine-5'-diphosphate (dUDP) and 2'-deoxycytidine-5'-diphosphate (dCDP), respectively (Hatse et al., 1999). Due to its relatively low enzymatic activity, RR-catalyzed 2'-deoxyribonucleoside-5'-diphosphate (dNDP) synthesis is the rate-limiting step in the entire process of DNA synthesis (Takeda and Weber, 1981; Weber, 1983). The *de novo* formation of thymine (Thy) nucleotides solely occurs by methylation of 2'-deoxyuridine-5'-monophosphate (dUMP) by thymidylate synthase (TS) resulting in the formation of thymidine-5'-monophosphate (dTMP). In this reaction, 5,10-methylenetetrahydrofolate serves both as one-carbon donor and as reductant (Santi, 1980). Ultimately, after nucleotide kinase-mediated phosphorylation, the end products thymidine-5'-triphosphate (dTTP) and 2'-deoxycytidine-5'-triphosphate (dCTP) are substrates for DNA polymerization.

Salvage of pyrimidines

Various pyrimidine salvage enzymes metabolize pre-existing pyrimidine nucleosides and nucleobases to meet the cellular nucleotide requirements. The salvage pathway therefore provides an alternative to the *de novo* pathway. As visualized in Figure 1, Thy and uracil (Ura) may be recycled in a reversible reaction catalyzed by thymidine phosphorylase (TP) to generate thymidine (dThd) and 2'-deoxyuridine (dUrd), respectively (Friedkin and Roberts, 1954; el Kouni et al., 1993). Both dThd and dUrd are substrates for thymidine kinase (TK)-mediated phosphorylation resulting in the formation of dTMP and dUMP, respectively. Similarly, Ura may be a source of UMP after conversion into uridine (Urd) by uridine phosphorylase (UP) and subsequent phosphorylation by uridine/cytidine (Urd/Cyd) kinase (Weber, 1983). Alternatively, mostly in prokaryotes, uracil may also be metabolized directly into UMP by a uracil phosphoribosyltransferase (UPRT). However, even though a presumed *UPRT* gene has been reported in several tissues of the human body, no catalytically competent *UPRT* has been detected in mammals so far (Li et al., 2007). *Cyd* is phosphorylated by Urd/Cyd kinase to yield cytidine-5'-monophosphate (CMP). Similarly, 2'-deoxycytidine (dCyd) is metabolized by 2'-deoxycytidine kinase (dCK) to 2'-deoxycytidine-5'-monophosphate (dCMP) which is a precursor of dUMP (upon deamination by the *de novo* enzyme *CMP/dCMP*-deaminase) and therefore of dTMP (Weber, 1983). *Cyd/dCyd* deaminase converts *Cyd* and *dCyd* to *Urd* and *dUrd*, respectively. In contrast to some prokaryotes, mammalian cells do not encode a cytosine deaminase and therefore catabolism of cytosine derivatives obligatorily occurs through deamination at the nucleoside level (Mullen et al., 1992).

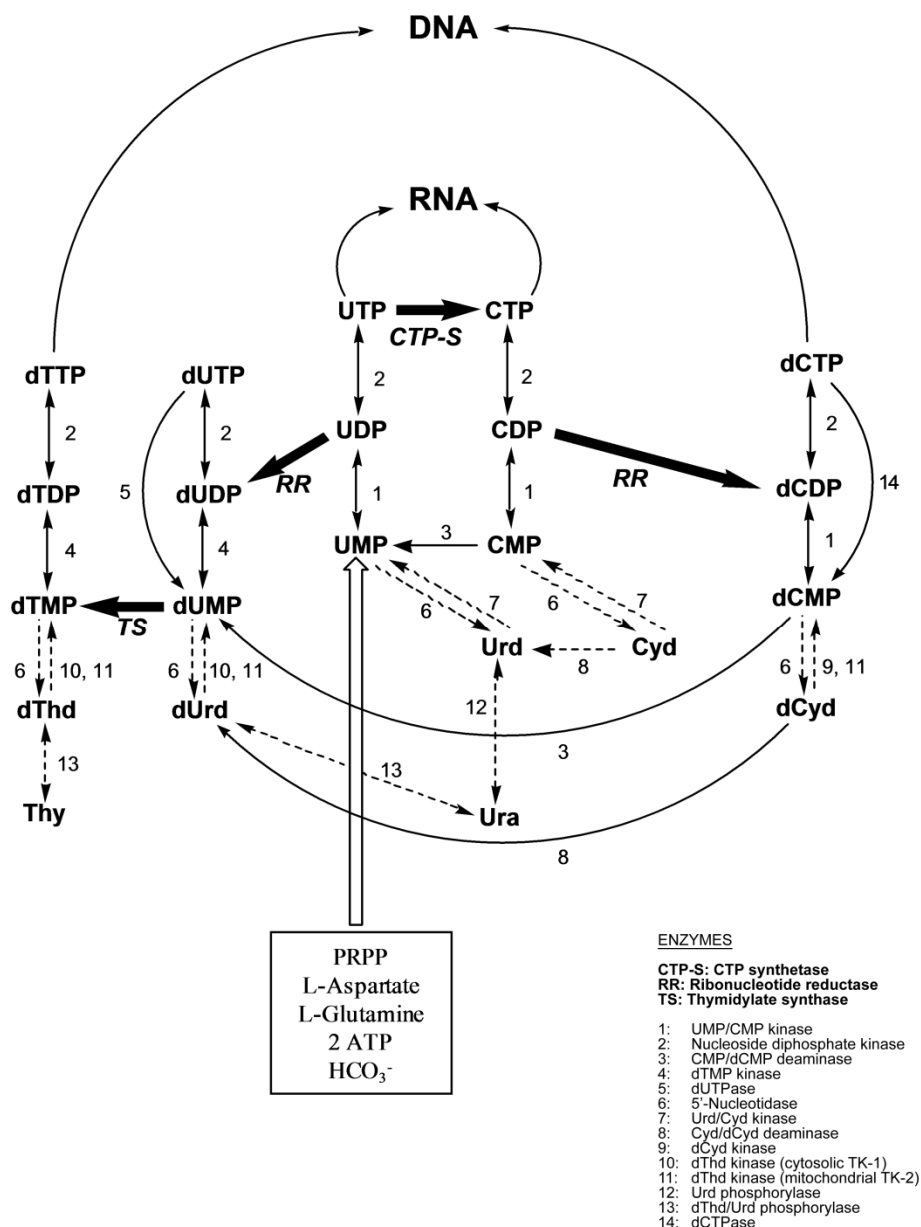


Figure 1: Schematic representation of the salvage and the *de novo* biosynthesis of pyrimidine nucleotides in mammalian cells. Dashed arrows represent enzyme activities that belong to the salvage pathway (modified from Hatse et al., 1999).

Purine nucleotide synthesis/metabolism

In purine nucleotide synthesis, inosine-5'-monophosphate (IMP) is the key nucleotide. IMP may be produced (i) by *de novo* synthesis, (ii) enzymatic conversion from AMP or GMP, or (iii) by salvage of hypoxanthine (Figure 2).

De novo synthesis of purine nucleotides

IMP is assembled from PRPP through 10 successive reactions. IMP may be converted into either adenosine-5'-monophosphate

(AMP) or guanosine-5'-monophosphate (GMP). The production of AMP from IMP, via the intermediate succinyl-AMP, requires energy derived from guanosine-5'-triphosphate (GTP). Conversely the production of GMP from IMP requires energy from ATP (Lieberman, 1956; Weber et al., 1992). This allows the cell to control the proportions of AMP and GMP. For GMP formation, IMP is converted into xanthosine-5'-monophosphate (XMP), in a rate-limiting reaction catalyzed by IMP

dehydrogenase (IMPDH), and subsequently converted into GMP by GMP synthetase (Weber, 1983). IMP may again be produced from AMP by AMP deaminase in the purine nucleotide cycle (Tornheim and Lowenstein, 1972) or from GMP by GMP reductase. Nucleotide kinase activity produces adenosine-5'-diphosphate (ADP) and ATP from AMP; and guanosine-5'-diphosphate (GDP) and GTP from GMP. The production of purine-based 2'-deoxyribonucleotides is catalyzed by RR from ADP and GDP resulting in the formation of 2'-deoxyadenosine-5'-diphosphate (dADP) and 2'-deoxyguanosine-5'-diphosphate (dGDP) which are further metabolized to produce 2'-deoxyadenosine-5'-triphosphate (dATP) and 2'-deoxyguanosine-5'-triphosphate (dGTP). IMP therefore eventually provides ATP and GTP for RNA synthesis and also dATP and dGTP for DNA synthesis (Hatse et al., 1999).

Salvage of purines

IMP, AMP and GMP may also be produced by alternative routes controlled by purine salvage enzymes (Figure 2). Purine nucleosides [inosine (Ino) and guanosine (Guo)] are efficiently catabolized into their respective nucleobases [hypoxanthine (Hx) and guanine (Gua)] in a reversible reaction catalyzed by purine nucleoside phosphorylase (PNP). Whereas mammalian PNP does not catalyze the conversion of adenosine (Ado) to adenine (Ade), prokaryotic PNP often also displays adenosine phosphorylase activity (Bzowska et al., 2000). Highly active phosphoribosyltransferases recycle nucleobases in the presence of PRPP directly to nucleotides. The conversion of Ade to AMP is catalyzed by adenine phosphoribosyl transferase (APRT) and Hx and Gua are efficiently metabolized to IMP and GMP respectively, by hypoxanthine-guanine phosphoribosyl transferase (HGPRT) (Hatse et al., 1999).

Nucleo(s)(t)ide metabolism in tumor cells

The presence of several rate-limiting enzymes in the *de novo* anabolic pathways of pyrimidine and purine metabolism en-

sure a controlled proliferative capacity in normal cells. In tumor cells, elevated activities of such enzymes [e.g. CTP synthetase, RR, TS, IMPDH and DNA polymerase] have been reported (reviewed by Weber, 1983; Hatse et al., 1999; Furuta et al., 2010). In addition, the activity of anabolic salvage enzymes such as dCK, TK, APRT and HGPRT were found to be increased in tumor cells whereas decreased activities of catabolic enzymes [e.g. dihydropyrimidine dehydrogenase (DHPD)] were observed. These alterations can be attributed to (i) mutations preventing normal allosteric enzyme inhibition as demonstrated for dATP-related inhibition of the R1 subunit of RR in murine lymphosarcoma cells (Weinberg et al., 1981), (ii) enzyme overexpression as observed for the R2 component of RR in premalignant breast lesions (Jensen et al., 1994a), IMPDH in several tumor cell types (Collart et al., 1992; Hubermann et al., 1994), or TS in metastatic melanoma (Vlaykova et al., 1997). Therefore, increased and imbalanced (2'-deoxy)nucleotide pools are often observed in tumor cells, leading to uncontrolled growth and increased mutation rates (de Korte et al., 1986; Meuth, 1989; Slingerland et al., 1994).

Therapeutic pyrimidine- and purine-based antimetabolites

The treatment of cancer is largely based on inhibition of cellular replication by cytostatic drugs. These include alkylating agents, oncolytic antibiotics, topoisomerase inhibitors, antimetabolites and others. In the early 1950s, the synthesis and cytostatic activity of purine nucleobase analogues was reported for the first time. Several studies demonstrated inhibitory activity of compounds such as 6-mercaptopurine (6-MP; Figure 3), 6-thioguanine (6-TG; Figure 3) and 2,6-diaminopurine against murine tumor cells *in vitro* and *in vivo*. Early clinical trials were performed and the administration of 6-MP to children with acute leukemia extended the median life expectancy of these patients to 12

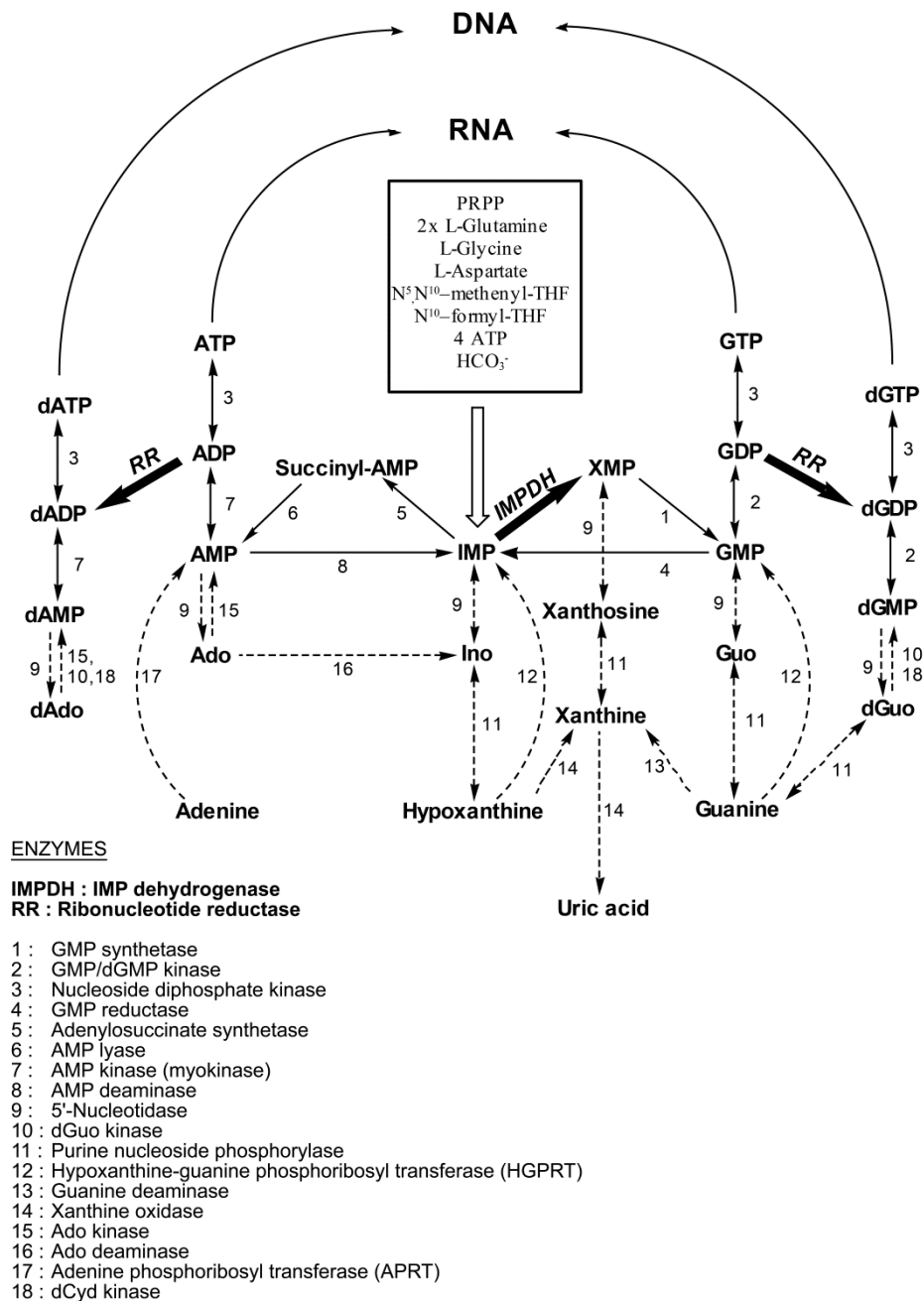


Figure 2: Schematic representation of the salvage and the *de novo* biosynthesis of purine nucleotides in mammalian cells. Dashed arrows represent enzyme activities that belong to the salvage pathway (modified from Hatse et al., 1999).

months compared with 3 - 4 months when treated with the antifolate methotrexate, being the mainstay treatment at that time. The FDA approval of 6-MP for the treatment of acute pediatric leukemia in 1953 was the start of extensive research on NAs as therapeutic agents (Elion, 1989). Cytarabine (cytosine-arabinofuranoside) was the first drug with a nucleoside-derived scaffold

that was approved by the FDA in 1969. This drug is used in the treatment of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) (Parker, 2009). Originally, NAs found their application in the treatment of hematological malignancies but currently a variety of pyrimidine and purine nucleoside derivatives also show activity towards several solid tu-

mors. With around 14 nucleobase/nucleoside-based antineoplastic agents being approved by the FDA for the treatment of different malignancies, nucleoside analogues (NAs) represent ~20 % of all clinically approved anticancer drugs (Table 1).

Three distinct classes of purine and pyrimidine antimetabolites can be distinguished based on their chemical structure (Figure 3): (i) thiopurines, (ii) fluoropyrimidines and (iii) 2'-deoxyribonucleoside analogues (Parker, 2009). The generalised mode of action of anticancer NAs is presented in Figure 4. Due to their similarity to the natural nucleic acid building blocks they are often internalized by cellular nucleoside- or nucleobase-specific transporter proteins and activated (e.g. phosphory-

lated) by previously discussed anabolic salvage enzymes to their 5'-monophosphate derivatives (Galmarini et al., 2002). The initial reaction is often rate-limiting in the activation of NAs and is mainly catalyzed by phosphoribosyl transferases (APRT or HGPRT) for nucleobase analogues; or kinases [e.g. dCK, TK and 2'-deoxyguanosine kinase (dGK)] for 2'-deoxyribonucleoside analogues (Elion, 1989; Johansson and Eriksson, 1996). Further phosphorylation to the 5'-diphosphate and 5'-triphosphate derivatives [catalyzed by nucleoside-5'-monophosphate kinases (NMPK) and nucleoside-5'-diphosphate kinases (NDPK), respectively] is required for most NAs to exert biological activity.

Table 1: FDA -approved purine and pyrimidine antimetabolites (modified from Liekens et al., 2009; Parker, 2009)

Drug	Main uses	FDA approval
5-aza-2'-deoxycytidine (decitabine)	Myelodysplastic syndromes	2006
O ⁶ -methylarabinofuranosyl guanine (nelarabine)	Acute lymphoblastic leukemia	2005
2'-fluoro-2'-deoxyarabinofuranosyl-2-chloroadenine (clofarabine)	Relapsed or refractory pediatric acute lymphoblastic leukemia	2004
5-aza-cytidine (vidaza)	Myelodysplastic syndromes	2004
N ⁴ -pentylloxycarbonyl-5'-deoxy-5-fluorocytidine (capecitabine)	Colorectal and metastatic breast cancer	1998
2',2'-difluoro-2'-deoxycytidine (gemcitabine)	Pancreatic, non-small cell lung, breast and bladder cancer	1996
2-chloro-2'-deoxyadenosine (cladribine)	Hairy-cell leukemia, non-Hogkin lymphoma	1992
arabinofuranosyl-2-fluoroadenine (fludarabine)	Chronic lymphocytic leukemia	1991
2'-deoxycoformycin (pentostatin)	Hairy-cell leukemia	1991
5-fluoro-2'-deoxyuridine (floxuridine)	Metastatic colorectal cancer	1970
arabinofuranosylcytosine (cytarabine)	Acute lymphoblastic and myeloid leukemia	1969
6-thioguanine	Acute lymphoblastic and myeloid leukemia	1966
5-fluorouracil	Gastrointestinal, pancreatic, head and neck, renal, skin and breast cancers	1962
6-mercaptopurine	Acute lymphoblastic leukemia	1953

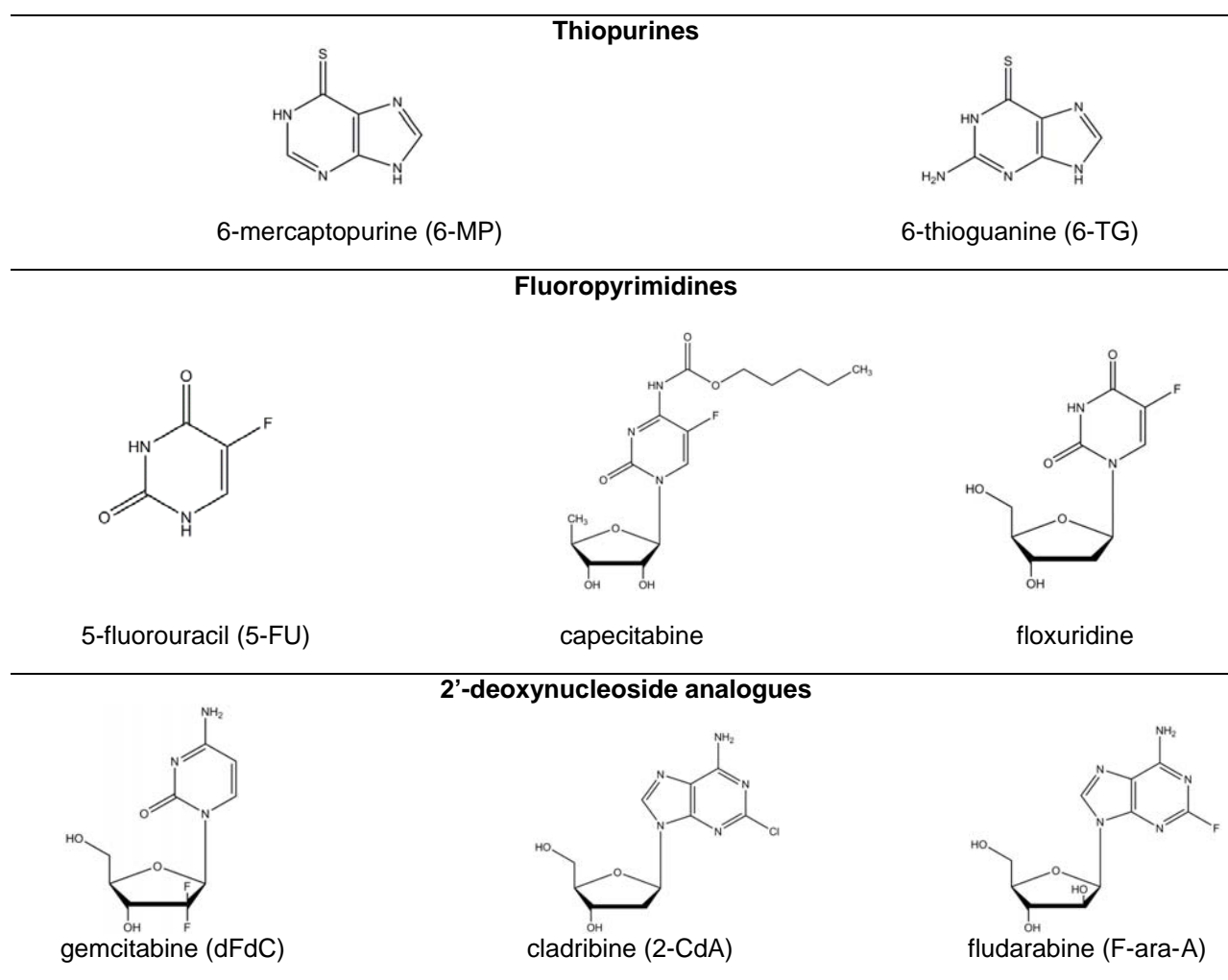


Figure 3: Chemical structures of representative NAs belonging to different classes of nucleobase/nucleoside analogues

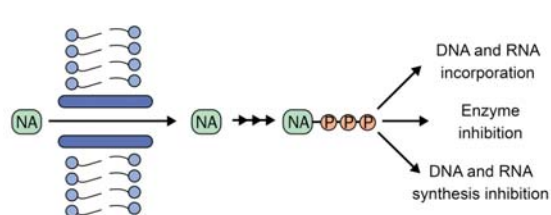


Figure 4: Schematic representation of the uptake, activation and mode of action of nucleobase/nucleoside analogues (modified from Galmarini et al., 2002).

Activated NAs may (i) inhibit one or several crucial enzymes in the *de novo* or salvage nucleo(s)tide metabolism or (ii) incorporate in nucleic acids. Both actions may lead to lethal DNA/RNA damage or inhibition of their synthesis, ultimately resulting in induction of apoptosis.

Enzymes responsible for the catabolism of pyrimidines and purines [e.g. DHPD and xanthine oxidase (XO), respectively] are often implicated in the detoxification of NAs (Galmarini et al., 2002). Most cells in adults are quiescent and therefore not actively proliferating. Targeting DNA synthesis may thus result in a certain level of selectivity towards tumor cells, although co-lateral toxicity mostly occurs in host tissues containing cells in their replicative state such as bone marrow, hair follicles, intestinal mucosa, etc. (Parker, 2009). The design of novel anticancer NAs focuses on drugs with a different mode of action or target or improved bioavailability/solubility. Several compounds

are currently under (pre)clinical investigation (reviewed by Jordheim et al., 2013).

Suicide gene therapy of cancer using non-mammalian enzymes

The eventual therapeutic efficiency of NAs is very much dependent on the expression and activity of nucleo(s)tide-metabolizing enzymes and their relative balances (activity rates) in tumor cells. Since non-mammalian enzymes (e.g. viral, prokaryotic and insect) are often endowed with a different substrate specificity or activity compared with their mammalian counterparts, the expression of such enzymes in tumor cells may alter NA-based treatment efficiency. In this respect, the selective introduction of non-mammalian genes in tumor cells to activate NA prodrugs has been investigated as a treatment modality for cancer. Such approaches are referred to as suicide gene therapy and the different gene/NA systems have been reviewed recently (Ardiani et al., 2012; Duarte et al., 2012). The therapeutic use of suicide gene therapy remains limited due to (i) inefficient gene delivery, (ii) poor catalytic conversion of nucleo(s)tide prodrugs by *wild-type* enzymes, (iii) toxicity caused by commensal prokaryotes in healthy tissues (e.g. intestinal) or (iv) thermostability of non-mammalian proteins at physiological temperature. In order to increase chemotherapeutic selectivity at the tumor site and to lower the required dose of prodrugs, research has focused on engineering more efficient mutant enzymes that differ in substrate specificity from both human and *wild-type* prokaryotic enzymes. Three illustrative gene therapy systems will be described.

Herpes simplex virus thymidine kinase (HSV-TK) phosphorylates the guanosine analogue ganciclovir (GCV) > 1000-fold more efficiently than cytosolic TK. Therefore, a selective phosphorylation of GCV to its 5'-monophosphate derivative (GCV-MP) may be obtained in HSV-TK-expressing tumor cells. GCV-MP is subsequently phosphorylated by endogenous en-

zymes (i.e. GMP kinase and NDPK) to produce GCV-5'-triphosphate which causes cell death after incorporation into the DNA. Successful results have been obtained with the HSV-TK/GCV system in several pre-clinical and some clinical studies. However its use remains limited due to the high doses of GCV that are required for therapeutic efficiency.

In contrast to mammalian cells, bacteria and yeast express a functional cytosine deaminase (CD) that catalyzes the hydrolytic deamination of the non-toxic prodrug 5-fluorocytosine (5-FC) to produce 5-fluorouracil (5-FU; Figure 3). 5-FU represents the mainstay of treatment for different malignancies. A CD/5-FC suicide gene therapy system has been explored using the *Escherichia coli* or *Saccharomyces cerevisiae* CD gene. Apart from selective activation of the drug at the tumor site, such system may be beneficial for some patients since 5-FC (and not 5-FU) may efficiently cross the blood-brain barrier (Vermes et al., 2000). Also, in contrast to the HSV-TK/GCV system, 5-FU (a small uncharged molecule) is not dependent on the occurrence of cell-to-cell contact or gap junctions to affect adjacent tumor cells (bystander effect) since the drug can pass the cell membrane by non-facilitated diffusion (Greco and Dachs, 2001).

Fludarabine (F-ara-A; Figure 3) and other adenosine analogues [e.g. 6-methylpurine-2'-deoxyriboside (6-MeP-dR) and 2-fluoro-2'-deoxyadenosine] have been studied extensively for their use against tumor cells, expressing *E. coli* PNP (reviewed in Zhang et al., 2005). Whereas mammalian PNP generally does not catalyze the phosphorolysis of (d)Ado and its analogues, prokaryotic PNP efficiently converts F-ara-A into its highly cytostatic base 2-fluoroadenine (2-FAde). 2-FAde may also diffuse to neighbouring tumor cells and inhibit RNA and/or protein synthesis when metabolized to 2-fluoro-ATP (by APRT-catalyzed ribophosphorylation and subsequent nucleotide kinase activity) (Hong et al., 2004).

Prokaryotic enzymes expressed at the tumor site due to a natural bacterial infection could also affect NA-based therapy. As will be discussed in detail below, bacteria belonging to the *Mycoplasmataceae* are a common source of mammalian cell culture contaminations and are reported to selectively colonize tumor tissue in cancer patients. This phenomenon is still greatly unrecognized and potentially underestimated but may have important implications in the optimization of current anticancer chemotherapy.

MYCOPLASMAS

General characteristics

In 1898 the contagious bovine pleuropneumonia (CBPP) agent, an important cause of lung disease in ruminants, was the first mycoplasma to be isolated and cultivated (Nocard and Roux, 1898). The term mycoplasma refers to its mycelar fungus-like morphology and CBPP was later identified as *Mycoplasma mycoides* subsp. *mycoides* small colony type (ter Laak, 1992). Indicative for its socioeconomic impact is the fact that CBPP is the only bacterial disease to be included in the A-list of the World Organization for Animal Health of prioritized communicable animal diseases (<http://www.oie.int>).

Bacteria from the genera *Mycoplasma*, *Ureaplasma* and *Allobaculum* belong to the family *Mycoplasmataceae* (trivial name: mycoplasmas) in the class of the *Mollicutes* [*mollis*, soft; *cutis*, skin (Latin)] which encompasses prokaryotes characterized by the lack of a rigid cell wall. Currently, at least 204 mycoplasmas are described of which 119 are classified under the genus *Mycoplasma* (Garrity et al., 2007). Mycoplasmas are the smallest (both in cellular dimensions and genome) and simplest self-replicating organisms. A variety of morphological entities were reported for mycoplasmas, with the predominant shape being a sphere with a diameter ranging from 0.3 - 0.8 μm . They are considered to have evolved from Gram-positive bacteria by degenerative evolution, including a

strong genome reduction and the loss of a rigid cell wall. These prokaryotes are surrounded by a plasma membrane only and therefore proved to be a valuable tool in membrane studies (Razin and Hayflick, 2010). Due to their small double stranded DNA genome (smallest \approx 580 kb), their metabolic activity is limited and mainly focused on energy generation. Mycoplasmas lack the genes for several biosynthetic metabolic pathways (e.g. the oxidative phosphorylation pathway and the tricarboxylic acid cycle) and have been studied extensively to define the minimal set of genes required for living organisms (the so-called minimal cell concept) (Razin et al., 1998; Glass et al., 2006).

Nucleo(s)(t)ide metabolism in mycoplasmas

The economization of genes in mollicutes is also reflected in their pyrimidine and purine metabolism and several interspecies variations in the biochemical pathways of mycoplasmas have been observed using enzymatic assays and comparative genomics (Bizarro and Schuck, 2007). Most mycoplasma species however share the inability to synthesize purine and pyrimidine nucleotides *de novo* (Mitchell and Finch, 1977; Pollack et al., 1997). Displaying a parasitic lifestyle, mycoplasmas therefore scavenge preformed nucleic acid precursors from their host tissue which are efficiently internalized and recycled using several transport mechanisms and salvage enzymes, respectively.

De novo biosynthesis of pyrimidine and purine nucleotides in mycoplasmas

As reviewed by Finch and Mitchell (1992), nutritional studies on the development of minimal growth media indicate a lack of *de novo* synthesis of pyrimidine and purine nucleotides in most mollicutes. The addition of yeast extract to culture media was shown to promote mycoplasma growth *in vitro*. This was partly attributed to its high content of nucleic acids (precursors). Later, it was shown that mycoplasma growth was also supported by a balanced

supply of DNA and RNA or by the addition of oligonucleotides. Further research revealed that the DNA synthesis requirements of mollicutes could also be met by the addition of dThd alone. Nucleic acid biosynthesis was studied extensively in *M. mycoides* subsp. *mycoides* for which a minimal growth medium was defined. Its simplest requirements for nucleic acid precursors were found to be Gua, Ura and Thy, which may be metabolized by a series of anabolic salvage enzymes. Using radio-labeled precursor studies, the absence of *de novo* synthesis was confirmed in most mycoplasma species (Mitchell and Finch, 1977; McElwain et al., 1988), *Mycoplasma penetrans* being a possible exception as indicated in a study by Sasaki and colleagues (2002). While other mycoplasmas lack either the gene encoding Urd/Cyd kinase or the gene encoding 5'-nucleotidase (involved in the salvage of UMP), both genes were found to be absent in *M. penetrans*. However, using comparative genomics, a set of 6 genes were identified in the genome of *M. penetrans*, encoding enzymes involved in an orotate-related pathway for the conversion of carbamoyl-phosphate to UMP. Since carbamoyl-phosphate is produced in the ATP-generating arginine dihydrolase pathway, this suggests that in *M. penetrans* UMP may be formed not only from Ura (by UPRT activity) but also using the *de novo* pathway.

It has long been assumed that most mollicutes lack a functional TS and therefore are unable to catalyze the methylation of dUMP to dTMP. As will be discussed later, dTMP was supposedly generated by salvage of Thy or dThd or by the uptake of dTMP from the environment (Pollack et al., 1997). However, TS activity has been described for *Mycoplasma pneumoniae* (Wang et al., 2010) and a flavin-dependent TS, without sequence homology to previously identified TS-encoding genes, was identified in *M. mycoides* and *Ureaplasma parvum*, the latter being a human pathogen colonizing the urogenital tract (Wehelie et al., 2010).

Salvage of pyrimidines and purines in mycoplasmas

Unlike the genome of their mammalian host (average human GC-content ~ 46 %), mycoplasma genomes are characterized by a high AT-content (70 - 75 %). In order to produce the required intracellular dTTP and dATP concentrations starting from the precursors supplied by their host, rearrangements of the internalized nucleobases/nucleo(s)(t)ides are required (Wang et al., 2001). Various mycoplasma-encoded catabolic and anabolic nucleotide salvage enzymes [e.g. nucleoside phosphorylases (NPs), nucleoside deaminases, nucleoside kinases, phosphoribosyltransferases, etc.] have been identified (Tham et al., 1993; Himmelreich et al., 1996) and mycoplasma infection was reported to upregulate host cell nucleoside catabolism and downregulate nucleoside anabolism *in vitro* (Johnson, 2008; Merkenschlager et al., 1988). Despite several inter-species variations, a general purine and pyrimidine salvage pathway for mycoplasmas has been outlined (Figure 5) (Finch and Mitchell, 1992; Pollack et al., 1997; Bizarro and Schuck, 2007).

As shown by Mitchell and Finch (1977) NP activity rapidly breaks down pyrimidine and purine nucleosides when supplied to mycoplasma cultures. As a result, (2'-deoxy)ribose-1-phosphate and the corresponding nucleobases are produced. High NP activity in mycoplasma-contaminated cell cultures has led to the design of assays to detect a mycoplasma infection *in vitro*. These assays are based on (i) decreased dTTP incorporation in infected host cells due to the presence of mycoplasma-encoded TP or (ii) Ado catabolism due to mycoplasma-encoded Ado phosphorylase (McGarrity and Carson, 1982; Jakway and Shevach, 1984; Sinigaglia and Talmadge, 1985; Merkenschlager et al., 1988).

Depending on the cellular requirements, nucleobases may be converted directly to their nucleoside-5'-monophosphate (NMP) derivatives by appropriate phos-

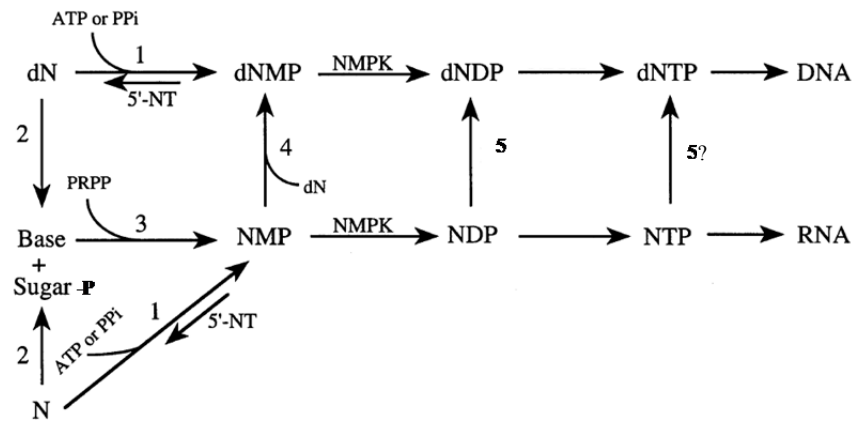


Figure 5: Schematic representation of potential pathways for nucleobase/nucleoside salvage in mycoplasmas (modified from Wang et al., 2001)

Enzymes: (1) ATP- or PP_i -dependent nucleoside kinases; (2) nucleoside phosphorylases; (3) phosphoribosyl transferases; (4) nucleoside phosphotransferases; (5) ribonucleotide reductase

Abbreviations: (d)N, (2'-deoxy)-nucleoside; (d)NMP, (2'-deoxy)nucleoside-5'-monophosphate; (d)NDP, (2'-deoxy)nucleoside-5'-diphosphate; (d)NTP, (2'-deoxy)nucleoside-5'-triphosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate; 5'-NT, 5'-nucleotidase; NMPK, nucleoside monophosphate kinase

phoribosyl transferases (UPRT, APRT or HGPR). Alternatively, (2'-deoxy)NMP [(d)NMP] may be produced from internalized (2'-deoxy)nucleosides by ATP-dependent or pyrophosphate(PP_i)-dependent nucleoside kinases (NKs). The presence of PP_i -dependent NKs is presumably valuable in reconstituting the required (2'-deoxy)nucleotides without consuming ATP (Tryon and Pollack, 1984; Wang et al., 2001). Some mycoplasmas may also replenish their deoxynucleotide pools by nucleoside phosphotransferase activity, transferring the 5'-phosphate moiety from a preformed NMP to 2'-deoxyribonucleosides (Wang et al., 2001). Also, while in most prokaryotic cells nucleotides are dephosphorylated by periplasmic phosphatases prior to uptake, mycoplasmas were shown to possess a single transporter system mediating the uptake of (d)NMPs. Exogenous supply of dTMP may therefore substitute for Thy when culturing mycoplasmas (Neale et al., 1984; Finch and Mitchell, 1992).

(d)NMPs are metabolized by sequential phosphorylation catalyzed by NMPK and NDPK. However, a gene encoding NDPK has not yet been identified in the genome of mycoplasmas and it has been hypothe-

sized that NDPK activity in mycoplasmas may be attributed to glycolytic kinases (such as pyruvate kinase) (Pollack et al., 2002), NMPK (Wang, 2007), or adenylate kinase (Bizarro and Schuck, 2007).

RR-catalyzed reduction of ribonucleotides to 2'-deoxyribonucleotides in mycoplasmas is believed to occur not only at the nucleoside-5'-diphosphate (NDP) level, as is observed in most organisms, but possibly NTPs are also directly reduced to generate dNTPs (Pollack, 2001). Since some mycoplasmas appear not to express a functional RR, these organisms may be fully dependent on deoxyribonucleoside salvage to meet their dNTP requirements (Glass et al., 2000).

Pathogenicity

Mycoplasmas are common parasites of mammals, plants, reptiles, arthropods and fish and were originally considered to be surface adherent (Figure 6). However, it is now generally accepted that several species (e.g. *M. pneumoniae*, *M. penetrans* and *Mycoplasma fermentans*, *M. genitalium* and *Mycoplasma hyorhinis*) may be internalized by the host cells, possibly shielding them from the host immune system and antibiotic treatment (Jensen et al., 1994b;

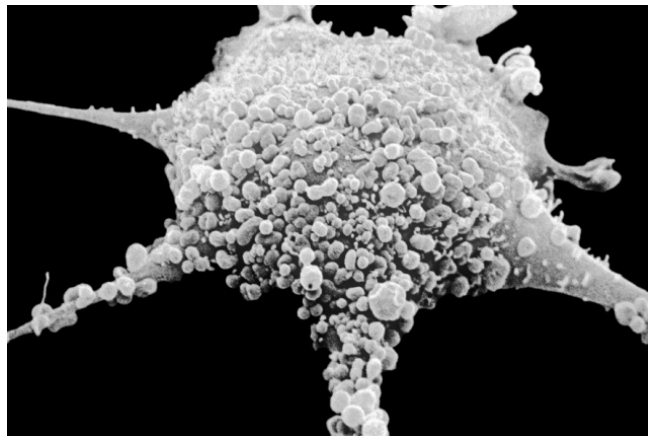


Figure 6: Scanning Electron Micrograph of *Mycoplasma* sp. on the surface of a fibroblast (© Dr. Phillips/Visuals Unlimited/Corbis)

Kornspan et al., 2010; Razin and Hayflick, 2010; Rottem et al., 2012). Due to their limited biosynthetic capabilities, mycoplasmas often exhibit a fairly strict host and tissue specificity.

In the human body, mollicutes mostly reside as commensals of the mucosal surfaces of the urogenital and respiratory tracts, often causing chronic asymptomatic infections (Razin et al., 1998). Transmission occurs by aerosol exposure or direct (intimate) contact between mucosal surfaces. Of healthy individuals studied, 84 % were determined to be a carrier of *Mycoplasma salivarium* or *Mycoplasma orale* in the oropharynx, and *Mycoplasma hominis*, *M. fermentans* and *Ureaplasma urealyticum* are frequently isolated from the urogenital tract (Krause and Taylor-Robinson, 1992). These data indicate that several mycoplasmas may be considered as being part of the resident flora of the human body. However, some species are also clearly associated with disease and the development of pathology may then depend on the specific site(s) of colonization, amount present, strain characteristics or lack of mucosal immunity (Krause and Taylor-Robinson, 1992). *U. urealyticum* is part of the genital flora of 45 - 75 % of the sexually active human population and infection is linked to the development of non-gonococcal urethritis (Murray et al., 2002). The most profoundly studied human myco-

plasma is *M. pneumoniae*, which was identified as the etiological agent of primary atypical pneumonia (Waites and Talkington, 2004). Using a special attachment organelle, this pathogen attaches to the surface of ciliary tracheal epithelia and hemolyses red blood cells by producing hydrogen peroxide. Furthermore, direct invasion of *M. pneumoniae* to extrapulmonary sites and/or induction of autoimmune response may result in severe, possibly life-threatening, complications. Due to their lack of a cell wall, mycoplasmas are resistant to treatment by cell wall-targeting antibiotics such as β -lactams (e.g. penicillin) but infections can be managed by the administration of macrolides, tetracyclines or fluoroquinolones (Waites and Talkington, 2004).

Additionally, several studies have investigated the link of mycoplasmas with a variety of diseases of unclear etiology (e.g. the Gulf War syndrome, chronic fatigue syndrome, Crohn's disease and others) (Baseman and Tully, 1997). However the specific association of mycoplasmas with these pathologies is doubtful (Razin, 1998). Also, a possible role for mycoplasmas as a co-factor of human immunodeficiency virus (HIV) infection and pathogenesis was studied extensively. *M. fermentans* and *M. penetrans* were frequently observed intracellularly in a variety of nonphagocytic cells in AIDS (acquired

immunodeficiency syndrome) patients but the relevance of AIDS-associated mycoplasmas remains unclear (Blanchard and Montagnier, 1994; Razin and Hayflick, 2010).

Mycoplasmas as cell culture contaminants

Studies have revealed that 15 - 80 % of the mammalian cell cultures worldwide are infected with mycoplasmas. Such contaminations may remain undetected for a long period of time as they do not produce the turbid growth that is characteristically observed in case of other bacterial or fungal infections in cell cultures (Drexler and Uphoff, 2002; Rottem et al., 2012). The main sources of contamination were identified as animal serum, trypsin, aerosols from previously infected cell cultures and laboratory staff (Drexler and Uphoff, 2002). Over 90 % of the contaminations were attributed to the human species *M. orale* and *M. fermentans* (presumably originating from laboratory manipulations); the bovine species *Mycoplasma arginini* (frequently isolated from bovine serum); and the swine pathogen *M. hyorhinis* (possibly transferred by trypsin which is/was obtained from swine pancreas) (Rottem and Barile, 1993; Razin and Hayflick, 2010). All cell types may be subject to mycoplasma contamination, however continuously propagated cell lines are more frequently infected compared with primary cell lines or those subcultivated for a limited time period. Contamination of primary cell lines is mostly due to an infection already present in the tissue from which the cell culture originated. Especially the foreskin, female urogenital tract and tumor tissues often show a high mycoplasma infection rate (Rottem et al., 2012).

Mycoplasmas adhere to their host cells via a process mediated by adhesins, accessory proteins and host receptors (reviewed by Rottem, 2003). Some mycoplasmas (e.g. *M. fermentans*) may fuse with their host cells when direct contact of the mycoplasma membrane with the cytoplasmic

membrane of the eukaryotic cell has been established. Mycoplasmal components (e.g. nucleases) may then be introduced into the host cell. Depending on the infected cell type and mycoplasma species or strain characteristics, host cells may display nutrient deprivation (e.g. amino acids and nucleic acid precursors), cytopathic effects and/or adherence or fusion-related damage. Also, several mycoplasmas (e.g. *M. penetrans*) may be internalized and remain viable within host cells. Intracellular multiplication of these invasive mycoplasma species has also been hypothesized (Rottem, 2003; Rottem et al., 2012).

Several methods for the detection and eradication of a mycoplasma infection in cell cultures have been described. Detection procedures include immunohistochemistry, electron microscopy, isolation and subsequent culturing assays, polymerase chain reaction (PCR) and various less specific assays. Elimination of the mycoplasmas may be achieved by physical (e.g. filtration), chemical (e.g. detergent exposure), immunological (e.g. *in vivo* passage through mice) and chemotherapeutic (e.g. antibiotics) eradication procedures. However, many of these assays have proven unreliable and/or time consuming and antibiotic treatment is by far the most common and efficient approach to remove mycoplasmas from the infected cell cultures (Drexler and Uphoff, 2002).

Mycoplasmas and cancer

According to the American Cancer Society, 15 - 20 % of all cancers worldwide are caused by infectious agents. The cancer-inducing mechanisms of several oncoviruses (e.g. human papillomavirus) are well documented and the link between specific bacterial infections (e.g. *Helicobacter pylori*) and the development of malignancy (e.g. gastric cancer) is generally accepted (zur Hausen, 2009; Touati, 2010). Also other prokaryotes (e.g. *Streptococcus sp.*, *Salmonella thyphii*, *Chlamydia sp.* and *Mycoplasma sp.*) are suggested to exhibit tumorigenic properties or may associate

with tumor tissue in cancer patients as opportunistic pathogens (reviewed by Vogelmann and Amieva, 2007; Cummins and Tangney, 2013). However, the oncogenic potential of mycoplasmas and their role in cancer development remains unclear.

Mycoplasmas and oncogenesis

In vitro studies have shown that prolonged exposure of cell cultures to different mycoplasmas (e.g. *M. fermentans*, *M. penetrans*, *M. genitalium*, *M. arginini* and *M. hyorhinis*) may induce chromosomal instability and malignant transformations, thereby promoting immortalization and characteristic tumor cell properties (i.e. increased migration, invasion and *in vivo* tumor formation) (Tsai et al., 1995; Zhang et al., 2004; 2006a; Namiki et al., 2009). The oncogenic potential of mycoplasmas may be due to a multistage process involving overexpression of oncogenes (e.g. *H-ras* and *c-myc*) (Zhang et al., 1997; 2006b), the increase of growth factor production (e.g. bone morphogenetic protein 2) (Jiang, et al., 2008), reduced activation of tumor suppressors (e.g. p53), and prevention of apoptosis (Feng et al., 1999; Logunov et al., 2008).

Extensive research has been done on p37, a *M. hyorhinis*-encoded surface protein, which is presumably part of a high-affinity transport system. Antibodies towards p37 were detected in the serum of cancer patients and isolation of the protein revealed that it originated from mycoplasmas (Fareed et al., 1988; Ketcham et al., 2005) The protein was also detected on the surface of FS9 mouse sarcoma cells and found to be responsible for their highly invasive behavior, which could be transferred to other cell lines by the addition of cell free FS9 supernatant. Mycoplasma contamination in these cell cultures was confirmed and elimination of the mycoplasmas or the addition of a specific anti-p37 antibody reduced invasiveness of the cells (Dudler et al., 1988; Schmidhauser et al., 1990). Subsequent *in vitro* studies have shown that p37 inhibits the adhesion and enhances motility, migration and invasion

of gastric and prostatic tumor cells (Ketcham et al., 2005; Liu et al., 2006). The expression of p37 in tumor cells was found to promote lung metastasis *in vivo* which was attributed to activation of matrix metalloproteinase-2 thereby inducing epidermal growth factor receptor (EGFR) (Gong et al., 2008). A protein with homology to p37 (Ag 243-5), was identified in *M. arginini* and was also shown to promote metastasis *in vivo* (Ushio et al., 1995). Therefore, it is hypothesized that mycoplasmas may stimulate tumor cell invasion and contribute to metastasis.

Association of mycoplasmas with established tumor tissue

Since the mid-1990s, the association between mycoplasmas and tumor tissue in cancer patients has been documented. Using different DNA-based or immunological detection assays, a high mycoplasma infection ratio was reported in several types of tumors (Table 2).

The first study reporting a high prevalence of mycoplasmas in tumor tissue was published by Sasaki et al. (1995), who found 48 % of gastric adenocarcinoma samples to be infected with *Mycoplasma sp.* (*M. hyorhinis* in 8 out of 11 positive samples). Yang et al. (2010) showed the presence of *M. hyorhinis* and *M. fermentans* in respectively 64 % and 31 % of resected gastric cancer tissues. Moreover, a higher mycoplasma infection ratio was observed in stage 3 - 4 samples compared with stage 1 - 2 samples and in diffuse-type compared with intestinal-type gastric carcinoma. Interestingly, lymph node metastasis was more evident in patients with mycoplasma-positive tumors (Yang et al., 2010). *Mycoplasma sp.* infections were also detected in 59 % and 21 % of ovarian cancer and cervical condyloma tissues, respectively (Chan et al., 1996; Kidder et al., 1998). However these studies did not include non-malignant control samples and therefore do not provide information on the preferential colonization of tumor tissue by mycoplasmas.

Table 2: Prevalence of mycoplasmas in tissue samples from cancer patients (cancerous, pre-cancerous or non-cancerous tissue) and from a control group (diseased or normal tissue)

Cancer type	Tissue from cancer patients			Tissue from control group		Species	Assay method	Reference
	Cancerous	Pre-cancerous	Non-cancerous	Diseased	Normal			
Gastric	48 % (n = 23)	-	-	-	-	<i>M. hyorhinis</i>	PCR	Sasaki et al., 1995
	56 % (n = 90)	-	-	28 – 37 % (n=142)	-	<i>M. hyorhinis</i>	IHC	Huang et al., 2001
	63.9 % (n = 61)	-	-	-	-	<i>M. hyorhinis</i>	nested PCR	Yang et al., 2010
	31.1 % (n = 61)	-	-	-	-	<i>M. fermentans</i>	nested PCR	Yang et al., 2010
Ovarian	59.3 % (n= 27)	-	-	-	-	unidentified	PCR/ELISA	Chan et al., 1996
Cervical	21.4 % (n = 14)	-	-	-	-	unidentified	PCR/ELISA	Kidder et al., 1998
Esophageal	50.9 % (n = 53)	-	-	-	-	<i>M. hyorhinis</i>	IHC	Huang et al., 2001
Lung	22.2 % (n = 27)	-	-	-	5 % (n = 40)	unidentified	PCR	Pehlivan et al., 2004
	52.6 % (n = 59)	-	-	-	-	<i>M. hyorhinis</i>	IHC	Huang et al., 2001
	100 % (n = 32)	-	-	-	-	unidentified	RT-PCR	Apostoulou et al., 2011
Breast	39.7 % (n = 63)	-	-	-	-	<i>M. hyorhinis</i>	IHC	Huang et al., 2001
Glioma	41 % (n = 91)	-	-	-	-	<i>M. hyorhinis</i>	IHC	Huang et al., 2001
Colon	55.1 % (n = 58)	-	-	20.9 % (n = 49)	-	<i>M. hyorhinis</i>	IHC	Huang et al., 2001
Prostate	22.4 % (n = 58)	13 % (n = 23)	-	6.8 % (n = 44)	0 % (n = 27)	<i>M. hominis</i>	PCR	Barykova et al., 2011
	54.8 % (n = 31)	53 % (n = 32)	-	20 % (n = 60)	0 % (n = 27)	<i>M. hominis</i>	qPCR	Barykova et al., 2011
	35 % (n = 31)	-	12.9 % (n = 31)	0 % (n = 31)	-	unidentified	PCR	Erturhan et al., 2013
RCC	82 % (n = 33)	67 % (n = 33)	36 % (n= 33)	-	15 % (n = 35)	unidentified	Nested PCR	Pehlivan et al., 2005

Huang et al. (2001) performed a large screening of different cancer tissues using immunohistochemistry and reported *M. hyorhina* infection rates of 40 - 53 % in esophageal, lung, breast cancer and glioma tissue samples. Importantly, increased mycoplasma infection ratios were detected in gastric carcinoma (56 %) compared with other gastric diseases (28 - 37 %, depending on the pathology), and colon carcinoma (55 %) compared with tissue derived from adenomatous polyps (21 %). Surprisingly, both for the gastric and colon carcinomas, the authors found *M. hyorhina* infection significantly increased in highly differentiated compared with poorly differentiated tissues whereas most studies report an increased mycoplasma infection in high *versus* low grade samples.

Mycoplasma infection was also compared in prostate biopsies from healthy individuals and patients with elevated prostate specific antigen (PSA) levels, indicative of benign prostatic hyperplasia (BPH), high-grade prostatic intraepithelial neoplasia (HGPIN; precursor for prostate cancer) and prostate cancer (Barykova et al., 2011). No mycoplasmas could be detected in the control group using PCR, whereas the presence of *U. urealyticum* (1 %), *M. genitalium* (6 %) and *M. hominis* (15 %) was demonstrated in samples derived from patients with elevated PSA levels. Moreover, *M. hominis* infection correlated with the severity of the diagnosis (7 %, 13 % and 22 % for BPH, HGPIN and cancerous tissue, respectively). In addition to an increased prevalence, a higher mycoplasma copy number (> 10-fold) was observed in *M. hominis*-positive prostate cancer or HGPIN compared with *M. hominis*-positive BPH tissue (Barykova et al., 2011). Patients with prostate cancer or HGPIN also showed increased seropositivity for *M. hominis* and *M. hyorhina* antibodies compared with individuals suffering from BPH (Barykova et al., 2011; Urbanek et al., 2011). A recent study also reported a significantly increased *Mycoplasma sp.* infection ratio in cancerous

tissue (35 %) compared with neighbouring benign tissue (13 %) in prostate biopsies from patients suffering adenocarcinoma of the prostate (Erturhan et al., 2013). In this study, no mycoplasmas were detected in prostate biopsies derived from patients suffering BPH.

A preferential colonization of the tumor site (82 %) compared with surrounding normal tissue (36 %) was also reported in 33 patients with conventional renal cell carcinoma (cRCC) (Pehlivan et al., 2005). Histological grading of the samples showed increased mycoplasma infection ratios in grade 3 - 4 samples compared with grade 1 - 2 samples. Moreover, cRCC patients without mycoplasmas were all characterized by low histological grade. However, there was no statistical difference between the mycoplasma prevalence in premalignant (renal intratubular neoplasia; 67 %) and malignant (82 %) tissue. Mycoplasmas were also detected in normal kidney tissue from healthy individuals, but at a much lower rate (15 %).

Pehlivan et al. (2004) reported a higher mycoplasma infection rate in lung tissue of patients suffering from small cell lung cancer (22 %) compared with tissue derived from a healthy control group (5 %). More recently, Apostoulou et al. (2011) found all assayed tissue samples that were surgically removed from lung cancer patients and analyzed for various prokaryotes and fungi to be positive for mycoplasmas. However, it seems that no samples derived from a control group were assayed so it cannot be excluded that this exceptionally high infection rate is due to external contamination of the samples or reagents.

The above-mentioned studies indicate an increased mycoplasma colonization of tumor tissue in cancer patients compared with (i) healthy tissue in the vicinity of the tumor site; (ii) non-malignant diseased tissue or (iii) healthy tissue derived from control patients. As suggested by Cummins and Tangney (2013), the preferential colonization of cancer cells by prokaryotes may be

attributed to an increased nutrient availability at the tumor site (e.g. due to necrosis) or bacterial chemotaxis. A possible explanation may also be found in a recent study by Huang and colleagues (2012) who described the role of the tumor suppressor protein PTEN (phosphatase and tensin homolog) in the cellular defense against bacterial infections. PTEN deficiency in mammalian cells conferred hyper-susceptibility to *Mycoplasma sp.* and *Mycobacterium bovis* infection. As various human cancers display mutations or deletions in PTEN, tumor cells could be more prone to infections by opportunistic pathogens such as mycoplasmas. However, the role and consequences of the presence of mycoplasmas at the tumor site remain to be elucidated and the above-mentioned studies have not been performed on a large cohort of patients. Also, apart from the study by Barykova et al. (2011), no mycoplasma quantification in tumors has been reported to date. Interestingly, most studies mentioned above report a high prevalence of *M. hyorhina* in human tumor tissue. However, the route of infection and relevance of this mycoplasma in the human body (*M. hyorhina* being a swine pathogen) remains to be investigated.

Mycoplasmas alter the cytostatic potential of several anticancer NAs

As mentioned above, several studies reported a decreased incorporation of dThd in different mycoplasma-infected cell cultures (Jakway and Shevach, 1984; Sinigaglia and Talmadge, 1985; Merckenschlager et al., 1988). This was attributed to enzymatic breakdown of exogenous pyrimidine nucleosides by mycoplasma-encoded NPs present in the supernatant of infected tumor cell cultures. Therefore, radiolabeled dThd incorporation was suggested as an assay for the detection of a mycoplasma contamination in cell cultures.

In contrast to mammalian cells and most prokaryotes, mycoplasmas were found to encode a single NP [i.e. a pyrimidine nu-

cleoside phosphorylase (PyNP)] for the catabolism of pyrimidine nucleosides (Vande Voorde et al., 2012). The expression of such PyNP in mycoplasma-infected cell cultures dramatically compromised the cytostatic activity of various pyrimidine-based NAs due to phosphorolysis of the drugs. The biological activity of compounds such as 5-FdUrd, 5-trifluorothymidine (TFT) and other halogenated dThd analogues was decreased 10- to 140-fold in mycoplasma-infected cell cultures due to PyNP-related conversion to less active (or inactive) nucleobases. Administration of the drugs along with a specific TP/PyNP inhibitor or elimination of the mycoplasmas using antibiotics rescued the cytostatic potential of these NAs (Bronckaers et al., 2008). Also, Jetté and colleagues (2008) found HCT116 colon cancer cell cultures to be 5- and 100-fold more resistant to 5-FU and 5-FdUrd, respectively, when contaminated with mycoplasmas.

Conversely, the activity of nucleoside-based drugs requiring phosphorolysis as an activation step was increased in mycoplasma-infected tumor cell cultures. The cytostatic potential of 5'-deoxy-5-fluorouridine (5'-DFUR), an intermediate metabolite of capecitabine, was increased >30-fold due to mycoplasma-encoded PyNP expression, efficiently converting the prodrug 5'-DFUR into its active metabolite 5-FU (Bronckaers, et al., 2008). Similarly, the non-toxic purine nucleoside analogue 6-MeP-dR was shown to severely affect mycoplasma-infected but not mycoplasma-free control tumor cell cultures. This was attributed to mycoplasma-encoded adenosine phosphorylase activity releasing the highly toxic 6-MeP from 6-MeP-dR. Recently, the *M. hyorhina* PNP was characterized and in contrast to its mammalian counterpart this enzyme also catalyzes the phosphorolysis of adenosine and different therapeutic purine nucleoside analogues including cladribine (2-CdA), Fara-A and 6-MeP-dR (Vande Voorde et al., 2013). Depending on the properties and metabolism of the nucleobase released upon

phosphorolysis of therapeutic NAs, a mycoplasma infection may therefore result in a decreased (as was observed for 2-CdA) or increased (as was observed for F-ara-A and 6-MeP-dR) cytostatic activity of the drugs. These biological effects can be normalized by the co-administration of mycoplasma-directed antibiotics or a specific PNP inhibitor.

Also, the cytostatic activity of the anti-cancer drug gemcitabine (used for the treatment of pancreatic, lung, breast and bladder cancer) was found to be decreased in mycoplasma-infected tumor cell cultures (Vande Voorde et al., 2009) due to the expression of a mycoplasma-encoded Cyd/dCyd deaminase (Vande Voorde, 2013). Preliminary *in vivo* studies confirmed a decreased anticancer effect of the drug in mycoplasma-infected mammary tumors in mice compared with uninfected control tumors (Vande Voorde, 2013).

CONCLUDING REMARKS

The presence of commensal bacteria has several implications in health and disease. Recent research revealed that an intact intestinal microbiome is required for the efficiency of immunotherapy or chemotherapy with platinum, doxorubicin or cyclophosphamide in tumor-bearing mice (Iida et al., 2013; Viaud et al., 2013). Several studies report the high and preferential colonization of different tumors by prokaryotes including mycoplasmas. Such mycoplasmas, present in the tumor microenvironment, may affect the efficiency of nucleoside-based antimetabolites. These findings argue for a careful consideration and possibly rational exploitation of the microbial target environment when optimizing nucleoside-based therapy (Liekens et al., 2009). It has been shown before that microbiota may influence the metabolism of NAs (Okuda et al., 1998; Parker, 2009). Metabolic phenotyping of body fluids or tissues has been proposed to predict the therapeutic outcome or potential toxicity of drugs (defined as pharmacometabonomics) (Clayton et al., 2006).

Such approach fits in the framework of precision medicine: the search for personalized healthcare by selection of appropriate targeted therapy based on biological information (either genetic, metabolic or physiologic). In this respect, the co-administration of a specific enzyme inhibitor or mycoplasma-directed antibiotic together with therapeutic NAs may prevent selective drug inactivation at the target site. Alternatively, a rational choice of drugs when treating mycoplasma-infected tumors could result in a more pronounced therapeutic selectivity. This may include (i) NP-insensitive derivatives of (approved) drugs (McGuigan et al., 2011; Vande Voorde et al., 2011) or (ii) compounds (i.e. prodrugs) that display an increased biological activity after phosphorolysis. An extensive study quantifying mycoplasmas in available tumor tissues and relating these findings to therapeutic outcome may reveal whether a pharmacometabonomic approach of nucleoside-based therapy could address inter-individual responses, improve drug efficacy and reduce adverse drug reactions.

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