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1. Introduction

Although research over the last decade has led to new and improved therapies for a variety of different diseases, anticancer drug therapy continues to have undesirable outcomes, including both poor response and severe toxicity. In addition to the critical need to discover new drugs, it is important to optimize existing therapies in order to minimize adverse reactions and maximize efficacy.

In the context of the complexity of cancer disease processes, future anticancer treatments will have to take into account the tumour microenvironment and aim to target the different cellular and molecular participants encompassed in a tumour, as well as their specific interactions.

In the present chapter we aimed to briefly summarize current knowledge on histamine and histamine receptors involvement in cancer, focusing on some recent evidence that points them out as a promising molecular targets and avenue for cancer drug development. On the basis of the role on immune system, it has been reported the efficiency of histamine as an adjuvant to tumour immunotherapy. In addition, we present here novel findings, suggesting the potential application of histamine and its ligands as adjuvants to tumour radiotherapy.

2. Histamine receptors

It is generally acknowledged that histamine is an important regulator of a plethora of (patho) physiological conditions and exerts its actions through the interaction with four histamine receptor subtypes. All these receptors belong to the family of heptahelical G-protein coupled receptors (GPCR) and they are the H₁, H₂, H₃ and H₄ histamine receptors (H₁R, H₂R, H₃R, H₄R). Based on the classical pharmacological analysis H₁R was proposed in 1966 by Ash and Schild (Ash & Schild, 1966) and H₂R was described in 1972 by Black et al. (Black et al., 1972). The third histamine receptor was discovered in 1983 by a traditional pharmacological approach, consisting of assessing the inhibitory effect of histamine on its own release from depolarized rat brain slices (Arrang et al., 1983). It was not until 2000-2001
that by using the H₃R DNA sequence, several independent research groups identified the novel H₄R highly expressed in immune cells (Coge et al. 2001b; Lui et al., 2001; Morse et al. 2001; Nakamura et al., 2000; Nguyen et al. 2001; Oda et al., 2000). Recent studies employing human genetic variance and mice lacking specific receptors or the ability to generate histamine, have shown functions for the histamine pathway that extend well beyond the established roles. As a result, antihistamines may have wider applications in the future than previously predicted (Smuda & Bryce, 2011).

Like most other GPCR, histamine receptors exist as equilibrium between their inactive and active conformations. Constitutive activity has now been shown for all four types of histamine receptors, leading to the reclassification of some antagonists as inverse agonists. These members of the GPCR family may exist as homo- and hetero-oligomers at the cell surface, which could have different pharmacological and physiological effects (Bongers et al., 2007; Fukushima et al., 1997; Hancock et al., 2003; Leurs et al., 2002, 2009). Moreover, the affinity of histamine binding to different histamine receptors varies significantly. Thus, the effects of histamine and receptor ligands upon receptor stimulation are rather complex. Pharmacologic agents are summarized in table 1.

### Table 1. Compounds most widely used in histamine receptor investigation

<table>
<thead>
<tr>
<th>Agonists</th>
<th>Antagonists/Inverse agonists</th>
</tr>
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<tbody>
<tr>
<td>H₁R</td>
<td>Histaprodifens, 2-(3-trifluoromethylphenyl)histamine</td>
</tr>
<tr>
<td>H₂R</td>
<td>Antimamine, impromidine, arpromidine</td>
</tr>
<tr>
<td>H₃R</td>
<td>R-(α)-methylhistamine, imetit, immepip</td>
</tr>
<tr>
<td>H₄R</td>
<td>Clobenpropit, VUF 8430, imetit, 4-methylhistamine, R-(α)-methylhistamine, OUP-16, clozapine</td>
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2.1 Histamine H₁R

Since histamine is considered to be the most important mediator in allergies such as allergic rhinitis, conjunctivitis, atopic dermatitis, urticaria, asthma and anaphylaxis, the most commonly used drugs to treat these pathological disorders are antihistamines acting on the H₁R. In the lung, it mediates bronchoconstriction and increased vascular permeability. The H₁R is expressed in a wide variety of tissues, including airway and vascular smooth muscle, endothelia, gastrointestinal tract, liver, genitourinary and cardiovascular systems, central nervous system (CNS), adrenal medulla, chondrocytes and in various immune cells including neutrophils, monocytes, eosinophils, dendritic cells (DC), as well as T and B lymphocytes, in which it mediates the various biological manifestations of allergic responses. The coding sequence of the human H₁R is intronless and is located in the chromosome 3 (Bakker et al., 2001; Dy & Schneider, 2004; Leurs et al., 1995). The human H₁R
contains 487 amino acids and is a Gq/11-coupled protein with a very large third intracellular loop and a relatively short C-terminal tail. The most important signal induced by ligand binding is the activation of phospholipase C (PLC)-generating inositol 1,4,5-triphosphate (Ins(1,4,5)P3) and 1,2-diacylglycerol leading to increased cytosolic calcium. In addition to the inositol signalling system, H1R activation could lead to additional secondary signalling pathways. This rise in intracellular calcium levels seems to account for the various pharmacological activities promoted by the receptor, such as nitric oxide production, vasodilatation, liberation of arachidonic acid from phospholipids and increased cyclic guanosine-3′,5′-monophosphate (cGMP). Additionally, it was reported that H1R can directly increase the cyclic adenosine-3′,5′-monophosphate (cAMP) levels (Davio et al., 1995). H1R also activates NF-kB through Gα11 and Gβγ upon agonist binding, while constitutive activation of NF-kB occurs only through the Gβγ (Bakker et al., 2001; Leurs et al., 1995; Smit et al., 1999). Recently, it was reported that the stimulation of H1R induced H1R gene expression through protein kinase C δ (PKCδ) activation, resulting in receptor upregulation (Mizuguchi et al., 2011).

2.2 Histamine H2R
The H2R principal action from a clinical point of view is its role in stimulating gastric acid secretion, thus H2R antagonists are used in the relief of symptoms of gastro-oesophageal reflux disease treatment. The human H2R intronless gene, encodes a protein of 359 amino acids and is located on chromosome 5. The H2R has a ubiquitous expression as the H1R. It is expressed in gastric parietal cells, heart, endothelial cells, nerve cells, airway and vascular smooth muscle, hepatocytes, chondrocytes and immune cells, such as neutrophils, monocytes, eosinophils, DC, and T and B lymphocytes (Black et al., 1972; Dy & Schneider, 2004; Leurs et al., 1995). The H2R is coupled both to adenylate cyclase via a GTP-binding protein Gs and phosphoinositide second messenger systems by separate GTP-dependent mechanisms. However, H2R-dependent effects of histamine are predominantly mediated by cAMP that activates protein kinase A (PKA) enzymes phosphorylating a wide variety of proteins involved in regulatory processes. Activation of H2R is also associated with other additional signal transduction pathways including activation of c-Fos, c-Jun, PKC and p70S6 kinase (Davio et al., 1995; Fitzsimons et al., 2002; Fukushima et al., 1997).

2.3 Histamine H3R
The H3R has initially been identified in both central and peripheral nervous system as a presynaptic receptor controlling the release of histamine and other neurotransmitters (dopamine, serotonin, noradrenalin, γ-aminobutyric acid and acetylcholine) (Arrang et al., 1983; Bongers et al., 2007; Leurs et al., 2005; Lovenberg et al., 1999). The H3R has gained pharmaceutical interest as a potential drug target for the treatment of various important disorders like obesity, myocardial ischemia, migraine, inflammatory diseases and several CNS disorders like Alzheimer’s disease, attention-deficit hyperactivity disorder and schizophrenia. Pitolisant (BF2.649, 1-[3-[3-(4-chlorophenyl)propoxy]propyl] piperidine, hydrochloride) is the first H3R inverse agonist to be introduced in the clinics. Its wake-promotion activity was evidenced in excessive diurnal sleepiness of patients with narcolepsy, Parkinson’s disease or obstructive sleep apnea/hypopnea (Bongers et al., 2007; Lebois et al., 2011; Leurs et al., 2005; Schwartz, 2011). The human H3R gene consists of either three exons and two introns, or four exons and three introns spanning 5.5 kb on
chromosome 20. Alternatively, the most 3' intron has been proposed to be a pseudo-intron as it is retained in the hH3R(445) isoform, but deleted in the hH3R(413) isoform. Overall similarity between the H3R and the H1R and H2R amounts to only 22% and 20%, respectively (Bongers et al., 2007; Coge et al., 2001a; Dy & Schneider, 2004; Leurs et al., 2005; Tardivel-Lacombe et al., 2001; Wellendorph et al., 2002).

The cloning of the human H3R has led to the discovery of many H3R isoforms generated through alternative splicing of the H3R mRNA. H3R can activate several signal transduction pathways, including Gi/o-dependent inhibition of adenylate cyclase that leads to inhibition of cAMP formation, activation of mitogen activated protein kinase pathway (MAPK), phospholipase A2, and Akt/protein kinase B, as well as the inhibition of the Na+/H+ exchanger and inhibition of K+-induced Ca2+ mobilization (Bongers et al., 2007; Coge et al., 2001a; Leurs et al., 2005; Wellendorph et al., 2002). A negative coupling to phosphoinositide turnover in the human gastric cell line HGT has also been described (Cherifi et al., 1992). Moreover, at least 20 isoforms of the human H3R have been described and they vary in the length of the third intracellular loop, their distinct CNS localization, differential signalling pathways and ligand binding affinity, which contribute to the heterogeneity of H3R pharmacology (Bongers et al., 2007; Coge et al., 2001a; Hancock et al., 2003; Leurs et al., 2005).

2.4 Histamine H4R

The identification by genomics-based approach of the human H4R by several groups has helped refine our understanding of histamine roles. It appeared to have a selective expression pattern restricted to medullary and peripheral hematopoietic cells including eosinophils, mast cells, DC, T cells and monocytes. Therefore, growing attention is directed towards the therapeutic development of H4R ligands for inflammation and immune disorders. Several lines of evidence suggest a role of the H4R in chronic inflammatory skin disease and the H4R might be a therapeutic target for diseases such as atopic dermatitis (Gutzmer et al., 2011). In addition, H4R was reported to be present on other cell types including intestinal epithelium, spleen, lung, stomach, CNS, nerves of nasal mucosa, enteric neurons and interestingly in cancer cells (Cianchi et al., 2005; Coge et al. 2001b; Connelly et al., 2009; Leurs et al. 2009; Lui et al., 2001; Medina et al., 2006; Morse et al. 2001; Nakamura et al., 2000; Nguyen et al. 2001; Oda et al., 2000). The significance of the H4R presence in various human tissues remains to be elucidated and therefore, new roles of H4R are still unrevealed (Leurs et al., 2009; Zampeli & Tiligada, 2009). The H4R cDNA was finally identified in the human genome database on the basis of its overall homology (37%, 58% in transmembrane regions) to the H3R sequence and it has a similar genomic structure. On the other hand, the homology with H1R and H2R is of approximately 19%. The human H4R gene that mapped to chromosome 18 is interrupted by two large introns and encodes a protein of 390 amino acids (Coge et al., 2001b; Leurs et al., 2009). H4R is coupled to Gai/o proteins, inhibiting forskolin-induced cAMP formation (Nakamura et al., 2000; Oda et al., 2000). Additionally, stimulation of H4R leads to activation of MAPK and also increased calcium mobilization via pertussis toxin-sensitive pathway (Leurs et al., 2009; Morse et al., 2001).

Isoforms have been described for the H4R which have different ligand binding and signalling characteristics. H4R splice variants [H4R (67) and H4R (302)] have a dominant negative effect on H4R (390) functionality, being able to retain it intracellularly and to inactivate a population of H4R (390) presumably via hetero-oligomerization (Leurs et al.,
In addition, H₄R dimeric structures that include homo- and hetero-oligomer formation and post-translational changes of the receptor might contribute to added pharmacological complexity for H₄R ligands (Leurs et al., 2009; van Rijn et al., 2006, 2008).

### 3. Histamine receptors in breast cancer

An estimated 1 million cases of breast cancer are diagnosed annually worldwide. Breast cancer is the most common neoplastic disease in women, and despite advances in early detection, about 30% of patients with early-stage breast cancer have recurrent disease, which is metastatic in most cases and whose cure is very limited showing a 5-year survival rate of 20% (Ferlay et al., 2010; Gonzalez-Angulo et al., 2007).

Histamine plays a critical role in the pathologic and physiologic aspects of the mammary gland, regulating cell growth, differentiation and functioning during development, pregnancy and lactation. Among monoamines, histamine demonstrates the greatest proliferative activity in breast cancer (Davio et al., 1994; Malinski et al., 1993; Wagner et al., 2003). Furthermore, histamine is increased in plasma and cancerous tissue derived from breast cancer patients compared to healthy group which is associated to an enhanced histidine decarboxylase (HDC) activity and a reduced diaminooxydase (DAO) activity that determine an imbalance between the synthesis and degradation of this monoamine. Histamine plasma level is dependent on the concentration of histamine in the tissues of ductal breast cancers, suggesting the participation of this monoamine in the development of this neoplasia (Reynolds et al., 1998; Sieja et al., 2005; von Mach-Szczyńska et al., 2009). A pilot study revealed that in samples of the same invasive ductal carcinoma patient, histamine peripheral blood levels tended to be reduced post-operatively (Kyriakidis et al., 2009). It was reported that in experimental mammary carcinomas, histamine becomes an autocrine growth factor capable of regulating cell proliferation via H₁R and H₂R, as one of the first steps responsible for the onset of malignant transformation. In this light, the in vivo treatment with H₂R antagonists produced the complete remission of 70% of experimental tumours (Cricco et al., 1994; Davio et al., 1995; Rivera et al., 2000). Many reports indicate the presence of H₁R and H₂R in normal and malignant tissues as well as in different cell lines derived from human mammary gland. H₂R produced an increase in cAMP levels while H₁R was coupled to PLC activation in benign lesions. On the other hand, H₁R was invariably linked to PLC pathway but H₂R stimulated both transductional pathways in carcinomas (Davio et al., 1993, 1996). However, the clinical trials with H₂R antagonists demonstrated controversial results for breast cancer (Bolton et al., 2000; Parshad et al., 2005).

Recently, it was demonstrated that H₃R and H₄R are expressed in cell lines derived from human mammary gland (Medina et al., 2006). Histamine is capable of modulating cell proliferation exclusively in malignant cells while no effect on proliferation or expression of oncogenes related to cell growth is observed in non-tumorigenic HBL-100 cells (Davio et al., 2002; Medina et al., 2006). Furthermore, histamine modulated the proliferation of MDA-MB-231 breast cancer cells in a dose-dependent manner producing a significant decrease at 10 μmol.L⁻¹ concentration whereas at lower concentrations increased proliferation moderately. The negative effect on proliferation was associated to the induction of cell cycle arrest in G2/M phase, differentiation and a significant increase in the number of apoptotic cells (Medina et al., 2006; Medina & Rivera, 2010b). Accordingly, by using pharmacological tools, results demonstrated that histamine increased MDA-MB-231 cell proliferation and also
migration via H3R. In contrast, clobenpropit and VUF8430 treatments significantly decreased proliferation. This outcome was associated to an induction of apoptosis determined by Annexin-V staining and TdT-mediated UTP-biotin Nick End labelling (TUNEL) assay, which was blocked by the specific H4R antagonist JNJ7777120. Also H4R agonists exerted a 2.5-fold increase in the cell senescence while reduced migration (Medina et al., 2008, 2010c, 2011b). Furthermore, histamine differentially regulates expression and activity of matrix metalloproteinases, cell migration and invasiveness through H2R and H4R in MDA-MB-231 cells modulating H2O2 intracellular levels (Cricco et al., 2011).

In addition, histamine at all doses tested, decreased the proliferation of a more differentiated breast cancer cell line, MCF-7, through the stimulation of the four histamine receptor subtypes exhibiting a higher effect through the H4R. Treatment of MCF-7 cells with the H4R agonists, inhibited cell proliferation and increased apoptosis and senescence (Medina et al., 2011b). These results represent the first report about the expression of H3R and H4R in human breast cells and interestingly show that the H4R is involved in the regulation of breast cancer cell proliferation, apoptosis, senescence, migration and invasion.

Recent results obtained with the orthotopic xenograft tumours of the highly invasive human breast cancer line MDA-MB-231 in immune deficient nude mice indicate that the H4R was the major histamine receptor expressed in the tumour. Remarkably, in vivo JNJ7777120 treatment (10 mg.kg−1, p.o., daily administration) significantly decreased lung metastases, indicating that H4R may be involved in the metastatic process (Medina & Rivera, 2010b). In addition, in vivo clozapine treatment (1 mg.kg−1, s.c., daily administration) significantly decreased tumour growth while enhanced survival of bearing tumour mice (Martinel Lamas et al., unpublished data).

Recent data indicate that H3R and H4R are expressed in human biopsies of benign lesions and breast carcinomas being the level of their expression significantly higher in carcinomas, confirming that H3R and H4R are present not only in cell lines but also in the human breast tissue. Furthermore, the expression of H3R is highly correlated with proliferation and histamine production in malignant lesions while the 50% of malignant lesions expressed H4R, all of them corresponding to metastases or high invasive tumours (Medina et al., 2008). The identification of histamine receptor subtypes and the elucidation of their role in the development and growth of human mammary carcinomas may represent an essential clue for advances in breast cancer treatment. The presented evidences contribute to the identification of molecules involved in breast carcinogenesis and confirm the role of H4R in the regulation of breast cancer growth and progression representing a novel molecular target for new therapeutic approach.

4. Histamine receptors in lymphomas and leukaemia

There is increasing evidence that histamine plays a role in cell differentiation and proliferation in several of normal tissues and in a wide range of tumours, including haematological neoplasias.

After an initial work in the late 1970s showing that histamine is able to induce haematopoietic stem cell proliferation via H2R (Byron, 1977), a real rush broke out in searching for further effects of histamine in haematopoiesis and haematological neoplasias. The histamine levels were determined in lymph nodes of patients with malignant lymphomas, Hodking’s disease (HD) or non-Hodking lymphomas (NHL), and in all cases the values were higher than in controls. In patients with NHL, these levels showed
Histamine Receptors as Potential Therapeutic Targets for Cancer Drug Development

dependence on the grade of malignancy as they found to be significantly higher in those classified as high-grade malignant (Belcheva & Mishkova, 1995). Immunostaining and ELISA method also confirmed the presence of histamine in the cytoplasm of acute lymphocytic leukaemia (ALL) cells, and H1R antihistamines inhibited their clonogenic growth. There was no correlation between the clonogenic growth of ALL cells and their histamine content, suggesting that while histamine may be important for the clonogenic growth of ALL cells; other factors also affect their clonogenity (Malaviya et al., 1996). Furthermore, leukaemia cell lines such as U937, expressed histamine receptors and a switch of histamine receptor expression from H2R to H1R during differentiation of monocytes into macrophages is observed (Wang et al., 2000).

Most patients with acute myeloid leukaemia (AML) achieve complete remission after induction chemotherapy. Despite ensuing courses of consolidation chemotherapy, a large fraction of patients will experience relapses with poor prospects of long-term survival. Interleukin-2 (IL-2) and interferon-alpha (IFN-alpha) are effective activators of lymphocytes with anti-neoplastic properties, such as T-cells or natural killer (NK) cells, constituting the basis for their widespread used as immunotherapeutic agents in human neoplastic disease. The functions of intratumoural lymphocytes in many human malignant tumours are inhibited by reactive oxygen species (ROS), generated by adjacent monocytes/macrophages. In vitro data suggest that those immunotherapeutic cytokines only weakly activate T cells or NK cells in a reconstituted environment of oxidative stress and inhibitors of ROS formation or ROS scavengers synergize with IL-2 and IFN-alpha to activate T cells and NK cells. Recently, IL-2 therapy for solid neoplastic diseases and haematopoietic cancers has been supplemented with histamine dihydrochloride (Ceplene), a synthetic derivative of histamine, with the aim of counteracting immunosuppressive signals from monocytes/macrophages. Histamine dihydrochloride inhibits the formation of ROS that suppress the activation of T cells and NK cells by suppressing the activity of NADPH oxidase via H2R. When administered in addition to IL-2, histamine dihydrochloride enables the activation of these lymphocytes by the cytokine, resulting in tumour cell killing. This combination was recently approved within the EU as a remission maintenance immunotherapy in AML, as histamine dihydrochloride reduces myeloid cell-derived suppression of anti-leukemic lymphocytes, improving NK and T-cell activation. Further research in this area will shed light on the role of histamine with the aim to improve cancer immunotherapy efficacy (Hellstrand et al., 2000; Martner et al., 2010; Yang & Perry, 2011).

5. Histamine receptors in gynaecologic cancers

Gynaecologic cancers encompass a remarkably heterogeneous group of tumours: cervical, ovarian, uterine, vaginal, and vulvar cancer. It has been postulated that histamine plays a critical role in proliferation of normal and cancer tissues, including the mammary gland, ovarian and endometrium.

In the murine uterus, the rapidly dividing epithelial cells of the endometrium can be defined as the major sources of histamine. In these cells the level of HDC expression is controlled mainly by progesterone-mediated signals which, interestingly, induce maximal level of HDC expression on the day of implantation (Pós et al., 2004).

In vitro studies showed that histamine may play an important role in follicular development and ovulation via H1R and H2R in women, acting as apoptosis inducer, taking part in the selection process of the dominant follicle and stimulating ovulation (Szukiewicz et al., 2007).
Interestingly, histamine content increased unequivocally in ovarian, cervical and endometrial carcinoma in comparison with their adjoining normal tissues, suggesting the participation of histamine in carcinogenesis. Besides, exogenous histamine, at micromolar concentration, stimulated proliferation of human ovarian cancer cell line SKOV-3 (Batra & Fadeel, 1994; Chanda & Ganguly, 1995). Preliminary results show that H₄R is expressed in primary and metastatic ovarian carcinoma and also in gallbladder cancer (Medina & Rivera, 2010b).

Histamine levels within ovarian tissue during the oestrus may correspond to cyclic changes of mast cells content and distribution in the ovary, suggesting an involvement of these cells in local regulation of ovarian function (Adyin et al., 1998; Nakamura et al., 1987). Interestingly, mast cells can typically be found in the peritumoural stroma of cervix carcinomas, as well as in many other cancers. Furthermore, high numbers of active, degranulated mast cells have been described in HPV infections and cervical intraepithelial neoplasias (Cabanillas-Saez et al., 2002; Demitsu et al., 2002). Hence, a functional relationship between mast cells and tumour cells has been proposed, where mast cells are involved in stimulating tumour growth and progression by enhancing angiogenesis, immunosuppression, mitogenesis, and metastasis (Chang et al., 2006). Mast cell activation leads to the release of inflammatory mediators, including histamine. Increased histamine levels have been described in the cervix lesions, where they have been associated with tumour growth and progression. Moreover, histamine receptors have been reported in different cell lines and tissues derived from experimental and human cervical neoplasias. The functional significance of immune cell infiltration of a tumour, specifically of mast cells located at the periphery of several neoplasias, is still a matter of controversy. Histamine acting via H₁R in cervical cancer cells could be pro-migratory, but when acting via H₄R could inhibit migration. On the other hand, other results also showed that cervical carcinoma cell mediators can activate mast cells to degranulate, demonstrating an active and dynamic cross-talk between tumour cells and infiltrating mast cells as shown in morphologic studies of neoplastic tissues (Rudolph et al., 2008).

In the light of these results, further investigations have to be done in order to elucidate the physiological role of histamine receptors on cell proliferation, as well as its implication in gynaecologic cancer progression with a potential interest for cancer treatment.

### 6. Histamine receptors in colorectal cancer

Colorectal cancer is one of the leading causes of cancer death among both men and women worldwide (Ferlay et al., 2010). It has been previously described that the histamine catabolising enzymes, DAO or histamine N-methyltransferase (HNMT), activities were significantly lower in adenoma tissue than in healthy mucosa in the same patients (Kuefner et al., 2008). Furthermore, HDC expression and its activity are increased in many human tumours including colorectal cancer (Cianchi et al., 2005; Masini et al., 2005; Reynolds et al., 1997). The levels of histamine were elevated in colon carcinoma and this is directly related to an increase in HDC expression and a decrease in DAO activity (Chanda & Ganguly, 1987). Also, the distribution of histamine receptors in the normal intestinal tract was reported (Sander et al., 2006). It was showed the expression pattern of H₁R, H₂R and H₄R in intestinal tract, receptors that were over expressed in the colon of patients with irritable bowel syndrome and food allergies. Furthermore, the H₃R was not detected in intestinal tissue (Sander et al., 2006). This data was further confirmed by Boer K et al, that also demonstrated
a decreased of H\textsubscript{1}R and H\textsubscript{4}R protein levels in colorectal cancer while the levels of the H\textsubscript{3}R were not modified compared to normal colon mucosa (Boer et al., 2008). It was described that the H\textsubscript{1}R antagonist, loratadine, inhibited proliferation and enhanced radiosensitivity in human colon cancer cells (Soule et al., 2010). Also the H\textsubscript{3}R seems to be implicated in the proliferation of colon cancer. In 1994 Adams, showed that \emph{in vivo} and in two human colonic adenocarcinoma cell lines, C170 and LIM2412, cell proliferation induced by histamine in a dose dependent manner was blocked by H\textsubscript{3}R antagonist, cimetidine (Adams et al., 1994). Ranitidine, another H\textsubscript{2}R antagonist, also showed to extend the survival of patients who were under surgery of colorectal cancer (Nielsen et al., 2002). It is well known the effects of histamine in the immune system, according to this it was demonstrated that patients receiving cimetidine or famotidine before curative resection augmented the probabilities of having tumour infiltrating lymphocytes in their tumours than control patients (Adams & Morris, 1996; Kapoor et al., 2005). Furthermore, earlier studies demonstrated that histamine induced \emph{in vitro} and \emph{in vivo} cell proliferation and this outcome was blocked by H\textsubscript{3}R antagonists (Adams et al., 1994; Cianchi et al., 2005). This effect was associated with the attenuation of anti-tumour cytokine expression in the tumour microenvironment exerted by histamine, thus resulting in stimulated colorectal cancer growth (Takahashi et al., 2001; Tomita & Okabe, 2005). In addition, H\textsubscript{3}R antagonist significantly suppressed the growth of tumour implants in mice by inhibiting angiogenesis via reducing VEGF expression (Tomita et al., 2003). As it was described above, the expression of the H\textsubscript{4}R seems to be suppressed in human colorectal cancer. It was also demonstrated that the levels of the H\textsubscript{4}R are reduced in advanced colorectal cancer compared with those in an initiating state, which suggest that the H\textsubscript{4}R expression is regulated during the progression of the disease (Fang et al., 2011). The stimulation \emph{in vitro} of the H\textsubscript{4}R by a specific agonist induced an augmented expression of the p21\textsuperscript{Cip1} and p27\textsuperscript{Kip1} proteins, producing an increase of arrested cells in the G1 phase. It has been proposed that prostaglandin E2 (PGE-2), the main product of the cyclooxygenase-2 activity, is implicated in colorectal cancer development. In this line, it has been demonstrated that histamine is fully implicated in the production of PGE-2 by its two receptors H\textsubscript{2}R and H\textsubscript{4}R in two human colon carcinoma cell lines (Cianchi et al., 2005). Histamine effect can be blocked by zolantidine, an H\textsubscript{3}R antagonist, and also by JNJ7777120, an H\textsubscript{3}R antagonist, whereas mepyramine, an H\textsubscript{1}R antagonist, has no effect on the production of PGE-2. Furthermore, JNJ7777120 inhibited the cell growth induced by histamine in three different human colon cancer cell lines and also inhibited the histamine-mediated increase in VEGF in two cell lines. Combined treatment with zolantidine (an H\textsubscript{3}R antagonist) and JNJ7777120 determined an additive effect on reducing the histamine-induced VEGF production and histamine-stimulated proliferation (Cianchi et al., 2005), suggesting the involvement of H\textsubscript{4}R in colon carcinogenesis (Boer et al., 2008).

7. Histamine receptors in melanoma

Malignant melanoma arises from epidermal melanocytes and despite being the cause of less than 5% of skin cancers, it is responsible for the large majority of skin cancer deaths (Ferlay et al., 2010). Early detection is vital for long-term survival, given that there is a direct correlation between tumour thickness and mortality (Cummins et al., 2006). Melanoma cells but not normal melanocytes contain large amounts of histamine that has been found to accelerate malignant growth (Pós et al., 2004). The absence of expression of
HDC in Mel-5 positive melanocytes isolated from skin samples of healthy persons, suggest that the level of HDC is strongly associated with malignancy in the skin (Haak-Frendscho et al., 2000). As a functional consequence of the inhibition of HDC protein synthesis, specific antisense oligonucleotide strongly (> 50%) decreased the proliferation rate of both WM938/B and HT168/91 human melanoma cells. Similar effects were found with other two melanoma cell lines WM35 and M1/15, suggesting that endogenous histamine may act as an autocrine growth factor (Hegyesi et al., 2000). On the other hand, overexpression of HDC markedly accelerated tumour growth and increased metastatic colony-forming potential along with rising levels of local histamine production that was correlated with tumour H2R and rho-C expression in mouse melanoma (Pós Z et al., 2005).

It has been previously reported the expression of H1R, H2R and H3R in melanoma cell lines (Hegyesi et al., 2005). In addition, it was described that in human melanoma cells, histamine acting through the H1R decreases cell proliferation, whereas it enhances growth when acting through the H2R (Lázár-Molnar et al., 2002). Furthermore, there is no evidence of mitogenic signalling through the H3R in human melanoma (Hegyesi et al., 2005).

H1R function is involved in chemotaxis via PLC activation, and its subsequent intracellular calcium mobilization. Proliferation assays showed that histamine exerted a concentration dependent dual effect on proliferation of the WM35 primary melanoma cell line. High concentrations of histamine (10^{-5} M) had an inhibitory effect while lower concentrations (10^{-7} M) increased colony formation. Similar results were achieved when using H1R agonist 2-(3-fluoromethylphenyl)histamine and H2R agonist arpromidine, respectively. The use of ranitidine, famotidine and cimetidine, all H2R specific antagonists, abolished the stimulatory effect of histamine on cell proliferation, indicating the participation of H2R in this mitogenic role of histamine. Second messenger measurement indicated that H2R are linked to cAMP production, thus suggesting an involvement of PKA in the mitogenic pathway triggered in this system, which is corroborated by the fact that forskolin and permeable cAMP analogues also produce a dose-dependent increase on cell proliferation (Lázár-Molnar et al., 2002).

Numerous *in vivo* studies employing animal models bearing syngenic or xenogenic melanoma grafts demonstrated that both endogenous and exogenous histamine have the ability to stimulate tumour growth while H2R antagonists (e.g. cimetidine, famotidine, roxatidine) inhibited this effect (Pós et al., 2005; Szincsák et al., 2002; Tomita et al., 2005; Uçar, 1991). Additionally, H2R antagonists stimulated melanogenesis and inhibited proliferation in B16-C3 mouse melanoma cells (Uçar, 1991). It was also found that melanoma tumour growth was not modulated by *in vivo* histamine treatment while treatment with terfenadine, an H1R antagonist, *in vitro* induced melanoma cell death by apoptosis and *in vivo* significantly inhibited tumour growth in murine models (Blaya et al., 2010).

Differences between melanoma cells in their capacity to produce and degrade histamine could explain the different sensitivities of melanoma cell types to exogenous histamine treatment. Moreover, there is evidence that cytokines can influence HDC expression and activity. It has been shown that there is a regulation loop between interleukin 6 (IL-6) and histamine: histamine increased IL-6 expression and secretion in metastatic lines via the H1R, and IL-6 treatment increased the HDC and histamine content in primary melanoma lines (Lázár-Molnar et al., 2002). Interferon-gamma (IFN-gamma) produced by surrounding immune cells decreases HDC expression, affecting melanoma growth and also impairs antitumour activity of the immune system, then contributing to the escape of melanoma cells from immunosurveillance (Horváth et al., 1999; Heninger et al., 2000). Furthermore,
mast cell activation initiates upon ultraviolet-B irradiation, which triggers histamine secretion acts as a cellular immunity suppressor (Chang et al., 2006). Moreover, the role of histamine in local immune reactions was further supported by the results of Hellstrand et al., who found that histamine can inhibit the ROS formation of monocytes/macrophages in the tumour (Hellstrand et al., 2000). This may explain the clinical benefit demonstrated by histamine (Ceplene) as an adjuvant to immunotherapy with IL-2 in several phase II and III clinical trials in metastatic melanoma (Agarwala, 2002). The addition of histamine dihydrochloride to an outpatient regimen of IL-2 is safe and well tolerated and demonstrates a survival advantage over IL-2 alone (9.4 vs. 5.1 months) in melanoma patients with liver metastases (Agarwala, 2002). However, a second confirmatory phase III study failed to show any survival benefit for those patients (Naredi, 2002).

Besides, Medina et al. showed that exogenous histamine modulated the activity of the antioxidant enzymes, increasing superoxide dismutase while decreasing catalase activity in WM35 melanoma cells. Accordingly, histamine treatment markedly augmented the levels of hydrogen peroxide and diminished those of superoxide anion, indicating that the imbalance of antioxidant enzymes leads to the cell proliferation inhibition (Medina et al., 2009). Furthermore, it was demonstrated that WM35 and M1/15 melanoma cells express H4R at the mRNA and protein level. By using histamine agonists and antagonists it was shown that the inhibitory effect of histamine on proliferation was in part mediated through the stimulation of the H4R. Treatment with a specific H4R antagonist, JNJ7777120 and the use of siRNA specific for H4R mRNA blocked the decrease in proliferation triggered by the H4R agonists. Furthermore, the decrease in proliferation exerted by H4R agonists was associated with a 2-fold induction of cell senescence and an increase in melanogenesis that is a differentiation marker on these cells (Massari et al., 2011). Current studies indicate that the H4R is expressed in the 42% of human melanoma biopsies of different histopathological types, showing cytoplasmic localization and confirming that the H4R is present not only in these cell lines but also in human melanoma tissue (Massari et al., 2011). The in vivo subcutaneous daily 1 mg.kg⁻¹ histamine or 1 mg.kg⁻¹ clozapine (H4R agonist) injections of M1/15 melanoma cell tumour bearing nude mice showed a survival increase vs. control group (treated with saline solution). Besides, results showed an antitumour effect of histamine and clozapine, including suppression of tumour growth (Massari et al., unpublished data). Further studies are needed to corroborate the H4R importance as potential target for new drug development for the treatment of this disease.

8. Histamine as a potential adjuvant to radiotherapy

8.1 Radioprotectors

Radiotherapy is the most common modality for treating human cancers and relies on ionising radiation induced DNA damage to kill malignant cells. Eighty percent of cancer patients need radiotherapy at some time or other, either for curative or palliative purpose. To optimise results, a cautious balance between the total dose of radiotherapy delivered and the threshold limit of the surrounding normal critical tissues is required. In order to obtain better tumour control with a higher dose, the normal tissues should be protected against radiation damage. Therefore, the role of radioprotective compounds is of utmost importance in clinical radiotherapy (Hall & Giaccia, 2006; Mah et al., 2011). Ionising radiation causes damage to living tissues through a series of molecular events. DNA double-strand breaks (DSBs), which are exceptionally lethal lesions, can be formed either by direct energy
deposition or indirectly through the radiolysis of water molecules, which generate clusters of ROS that react with DNA molecules. Because human tissues contain 80% water, the major radiation damage produced by low linear transfer energy (LET) radiation is due to the aqueous free radicals. DSBs are essentially two single stranded nicks in opposing DNA strands that occur in close proximity, severely compromising genomic stability (Grdina, 2002; Hall & Giaccia, 2006; Mah et al., 2011). A series of complex pathways collectively known as the DNA damage response (DDR) is responsible for the recognition, signalling and repair of DSBs in cells, ultimately resulting in either cell survival or cell death (Mah et al., 2011). These free radicals react not only with DNA but also with other cellular macromolecules, such as RNA, proteins, membrane, etc, and cause cell dysfunction and mortality. Unfortunately, these reactions take place in tumour as well as normal cells when exposed to radiation. Therefore, to improve the efficacy of radiotherapy there is an intense interest in combining this modality with ionising radiation modifiers, such as radioprotectors. These compounds mitigate damage to surrounding non-malignant tissue (Brizel, 2007; Grdina, 2002; Hall & Giaccia, 2006; Hosseinimehr, 2007).

The most remarkable group of true radioprotectors is the sulfhydryl compounds. The simplest is cysteine, a sulfhydryl compound containing a natural amino acid (Table 2). In 1948, Patt discovered that cysteine could protect mice from the effects of total-body exposure to X-rays if the drug was injected or ingested in large amounts before the radiation exposure. At about the same time, in Europe independently discovered that cysteamine could also protect animals from total-body irradiation (Table 2). However, cysteine is toxic and induces nausea and vomiting at the dose levels required for radioprotection. A developmental program was initiated in 1959 and conducted at the Walter Reed Institute of Research to identify and synthesize drugs capable of conferring protection to individuals in a radiation environment by the U.S. Army. Over 4,000 compounds were synthesized and tested and it was discovered that the covering of the sulfhydryl group by a phosphate group reduced toxicity (Grdina, 2002; Hall & Giaccia, 2006; Nucifora et al., 1972).

The concept of the therapeutic ratio is central to understanding the rationale for using radioprotectors. It relates tumour control probabilities and normal tissue complication probabilities to one another. An ideal radioprotector will reduce the latter without compromising the former and should also be minimally toxic itself. Radioprotective strategies can be classified under the categories of protection, mitigation, and treatment. Protectors are administered before radiotherapy and are designed to prevent radiation-induced injury. Amifostine is the prototype drug (Table 2). Amifostine is the only radioprotective agent that is approved by FDA for preventing of xerostomia induced by gamma irradiation in patients under radiotherapy (Grdina et al., 2009; Hall & Giaccia, 2006; Hosseinimehr, 2007; Kouvaris et al., 2007, Wasserman & Brizel, 2001). Its selectivity for normal tissue is due to its preferential accumulation in normal tissue compared to the hypoxic environment of tumour tissues with low pH and low alkaline phosphatase, which is required to dephosphorylate and activate amifostine (Calabro-Jones et al., 1985; Grdina, 2002; Mah et al., 2011). The active metabolite, WR-1065 scavenges free radicals and is oxidised, causing anoxia or the rapid consumption of oxygen in tissues. This sulfhydryl compound is one of the most effective radioprotectors known nowadays, but there are two main problems of its using. The first one is their toxicity and the second is the short-ranged activity. Amifostine is also the unique radioprotector widely used in clinic on chemotherapy applications (Grdina et al., 2009; Hall & Giaccia, 2006; Hosseinimehr, 2007).
<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>SIDE EFFECTS</th>
<th>CHEMICAL STRUCTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amifostine (WR-2721)</td>
<td>Drowsiness, feeling of coldness, flushing/feeling of warmth; hiccups, nausea, sneezing, vomiting</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Cysteamine</td>
<td>Depression, stomach or intestinal ulcer and bleeding, liver problems, skin condition, decreased calcification of bone, seizures, broken bone, decreased white blood cells</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Palifermin</td>
<td>Skin rash, flushing, unusual sensations in the mouth (tingling, tongue thickness)</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Cysteine</td>
<td>Toxic, nausea, vomiting</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Tempol</td>
<td>Constipation; diarrhoea, severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue), loss of appetite, muscle weakness, nausea, slow reflexes, vomiting</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
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Table 2. Radioprotectors. Extracted and modified from http://www.wolframalpha.com/entities/chemicals/palifermin/hs/j8/6k/; http://www.drugs.com

Mitigants are administered after radiotherapy but before the phenotypic expression of injury and are intended to ameliorate injury. The keratinocyte growth factor (KGF), palifermin, has been approved as a new, targeted therapy for the prevention of severe oral mucositis in patients with head and neck cancer undergoing post-operative radiochemotherapy and can be considered as the prototype mitigant (Weigelt et al., 2011) (Table 2). Palifermin, like the natural KGF, helps maintain the normal structure of the skin and gastrointestinal surface (lining) by stimulating cells to divide, grow and develop (Le et al., 2011; Weigelt et al., 2011).

Treatment is a strategy that is predominantly palliative and supportive in nature. Pharmacologic radioprotective strategies should be integrated with physical strategies such as intensity-modulated radiotherapy to realize their maximum clinical potential (Hall & Giaccia, 2006; Le et al., 2011).

In addition, low-to-moderate doses of some agents such as nitroxides, adrenoceptor agonist, were found to have radioprotective activity in experiments but their application in clinic remains doubtful. Tempol (4-hydroxy-2,2,6,6-tetramethyl-piperidinyloxy) belongs to a class of water-soluble nitroxides which are membrane-permeable stable free radical compounds that confer protection against radiation-induced damage (Bennett et al., 1987; Mah et al., 2011; Muscoli et al., 2003) (Table 2). It is thought to elicit its effects through the oxidation of reduced transition metals, scavenging free radicals and mimicking superoxide dismutase activity (Jiang et al., 2007).

### 8.2 Histamine as a radioprotector

Despite many years of research there are surprisingly few radiation protectors in use today, whose clinical value is limited due to their toxicity; thus, the development of effective and nontoxic agents is yet a challenge for oncologists and radiobiologists (Hall & Giaccia, 2006).
The acute effects of irradiation result from the death of a large number of cells in tissues with a rapid rate of turnover. These include effects in the epidermal layer or skin, gastrointestinal epithelium, and haematopoietic system, in which the response is determined by a hierarchical cell lineage, composed of stem cells and their differentiating offspring. In clinical radiotherapy, the tolerance of normal tissues for radiation depends on the ability of clonogenic cells to maintain a sufficient number of mature cells suitably structured to preserve organ function (Hall & Giaccia, 2006). During radiotherapy for intra-abdominal and pelvic cancers, radiation seriously affects radiosensitive tissues such as small intestine and bone marrow (Erbyl et al., 2005; Hall & Giaccia, 2006). It was previously demonstrated that histamine treatment (daily subcutaneous injection, 0.1 mg.kg\(^{-1}\)) significantly protects mouse small intestine against radiation-induced toxicity ameliorating histological injury and improving trophism of enterocytes (Medina et al., 2007). Histamine completely prevented the decrease in the number of crypts evoked by whole body irradiation, which is vital for small intestine restoration since the intestinal crypt contains a hierarchy of stem cells that preserve the potential to regenerate the stem cell population and the tissue after cytotoxic exposure (Potten et al., 2002). Histamine radioprotective effect on small intestine was related to an increased rate of proliferation as evidenced by the enhanced proliferation markers immunoreactivity [5-bromo-2′-deoxyuridine (BrdU), and proliferating cell nuclear antigen (PCNA)]. Additionally, this outcome was accompanied by a reduction in the number of apoptotic cells per crypt and a modification of antioxidant enzyme levels that could lead to enhance the antioxidant capacity of intestinal cells (Medina et al., 2007). Histamine also protects rat small intestine against ionising radiation damage and this effect was principally associated to a decrease in intestinal cell crypt apoptosis (Medina & Rivera, 2010a).

The bone marrow pluripotent stem cells, such as erythroblast, are particularly radiosensitive and, after whole body irradiation, an important grade of aplasia is observed increasing the possibility of haemorrhage and/or infection occurrence that could be lethal. The survival of stem cells determines the subsequent repopulation of bone marrow after irradiation (Hall & Giaccia, 2006). Results demonstrated that histamine (0.1 mg.kg\(^{-1}\)) significantly reduced the grade of aplasia, ameliorating the oedema and vascular damage produced by ionising radiation while eliciting a significant conservation of the medullar progenies on bone marrow in mouse and rat species, increasing the number of megakaryocytes, myeloid, lymphoid and erythroid cells per mm\(^2\). The histamine effect is mediated at least in part by an increase in the rate of proliferation, as evidenced by the enhanced PCNA protein expression and BrdU incorporation, and is associated with an enhanced HDC expression in irradiated bone marrow cells (Medina et al., 2010; Medina & Rivera, 2010a). In this line, it was reported that a faster bone marrow repopulation was observed in wild type in comparison with HDC-deficient mice and that intracellular HDC and histamine content in regenerating bone marrow populations is increased after total-body irradiation (Horvath et al., 2006).

Despite improvements in the technology for delivering therapeutic radiation, salivary glands are inevitably injured during head and neck cancer radiotherapy, causing devastating side-effects which results in salivary hypofunction and consequent xerostomia (Burlage et al., 2008; Hall & Giaccia, 2006; Nagler, 2002). Salivary glands of rat are quite similar to human salivary glands in which salivary flow is rapidly reduced after radiation exposure (Nagler, 2002). Recent results demonstrated that histamine markedly prevented radiation injury on submandibular gland, ameliorating the histological and morphological alterations. Radiation significantly decreased salivation by approximately 35-40%, which
was associated with a reduction of submandibular gland wet weight and an alteration of epithelial architecture, vacuolization of acinar cells and partial loss of eosinophilic secretor granular material. It is worth noting that histamine treatment (0.1 mg.kg\(^{-1}\)) completely reversed the reduced salivation induced by radiation, preserving glandular function and mass with normal structure organization of acini and ducts. Histamine prevented radiation-induced toxicity in submandibular gland essentially by suppressing apoptosis of ductal and acinar cells, reducing the number of apoptotic cells per field (Medina et al., 2011a).

To summarize, histamine treatment can selectively modulate cellular damage produced by ionising radiation, thus preventing radiation induced damage on small intestine, bone marrow and salivary glands. Furthermore, histamine \textit{in vitro} enhances the radiosensitivity of breast cancer cells (Medina et al., 2006) while does not modify that of melanoma (Medina et al., 2007). Despite histamine may be proliferative in some cancer cell types, it may still be beneficial as radioprotector in view of the fact that it is only administered for a short period of time to reduce the radiation induced damage. It is important to highlight that histamine radioprotective effect was demonstrated in two different rodent species, which suggests that histamine could exert a radioprotective action in other mammals. Also, no local or systemic side effects were observed upon histamine administration in both species.

The presented evidences indicate that histamine is a potential candidate as a safe radioprotective agent that might increase the therapeutic index of radiotherapy for intra-abdominal, pelvic, and head and neck cancers, and enhance patient quality of life by protecting normal tissue from radiation injury. However, the efficacy of histamine needs to be carefully investigated in prospective clinical trials.

9. Conclusions

In this chapter, we have presented major findings of the most recent research in histamine cancer pharmacology. These data clearly indicate that histamine plays a key role as a mediator in most human tumours. Interestingly, histamine is not only involved in cancer cell proliferation, migration and invasion, but also the tumour microenvironment and immune system responses are tightly affected. In human neoplasias H\(_3\)R and H\(_4\)R seemed to be the main receptors involved in the control of the metabolic pathways responsible for tumour growth and progression, suggesting that H\(_3\)R and H\(_4\)R represent potential molecular targets for cancer drug development. Finally, a novel role for histamine as a selective radioprotector is highlighted, indicative of the potential application of histamine and its ligands as adjuvants to radiotherapy.

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This book represents a case study based overview of many different aspects of drug development, ranging from target identification and characterization to chemical optimization for efficacy and safety, as well as bioproduction of natural products utilizing for example lichen. In the last section, special aspects of the formal drug development process are discussed. Since drug development is a highly complex multidisciplinary process, case studies are an excellent tool to obtain insight in this field. While each chapter gives specific insight and may be read as an independent source of information, the whole book represents a unique collection of different facets giving insight in the complexity of drug development.

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