### Title

Chapter Ten Hyaluronan–CD44 Interaction Promotes Oncogenic Signaling, microRNA Functions, Chemoresistance, and Radiation Resistance in Cancer Stem Cells Leading to Tumor Progression




### Authors

Bourguignon, Lilly YW
Shiina, Marisa
Li, Jian-Jian



### Publication Date

2014

### DOI

10.1016/b978-0-12-800092-2.00010-1

Peer reviewed

CHAPTER TEN

# Hyaluronan–CD44 Interaction Promotes Oncogenic Signaling, microRNA Functions, Chemoresistance, and Radiation Resistance in Cancer Stem Cells Leading to Tumor Progression

**Lilly Y.W. Bourguignon*,[1], Marisa Shiina*, Jian-Jian Li†**
*Department of Medicine, University of California at San Francisco & Endocrine Unit (111N), VA Medical Center, San Francisco, California, USA
†Department of Radiation Oncology, University of California Davis, Sacramento, California, USA
[1]Corresponding author: e-mail address: lilly.bourguignon@ucsf.edu

## Contents



## Abstract

Hyaluronan (HA), a major component of the extracellular matrix (ECM), is enriched in many types of tumors. There is good evidence linking high levels of HA production in human carcinomas to an aggressive phenotype and tumor metastasis. HA is generally bound to CD44 isoforms (so-called CD44s and CD44v3) which are ubiquitous, abundant, and functionally important cell surface receptors. This chapter describes the evidence

*Advances in Cancer Research*, Volume 123
ISSN 0065-230X
http://dx.doi.org/10.1016/B978-0-12-800092-2.00010-1

for HA/CD44v3-mediated activation of the cytoskeleton (e.g., ankyrin and GTPases) and matrix metalloproteinase (MMP) signaling during tumor progression.

A special focus is placed on the role of HA–CD44v3 interaction in cancer stem cells (CSCs). Matrix HA is known to be present in CSC niches. Since CD44v3 serves as a CSC marker, it provides an important physical linkage between matrix HA and various transcription factors that regulate tumor cell functions through distinct signaling pathways. CSCs are known to be chemoresistant and/or radiation resistant and to cause cancer relapse. The purpose of this chapter is to review the most current research on the cellular and molecular biology of CSCs. The emphasis will be placed on both CSC niche and matrix HA-induced microRNA signaling plus various CSC functions (e.g., self-renewal, differentiation, and chemoresistance) during cancer progression. Understanding the regulation of CSCs is critically important for designing CSC-specific therapeutic targets to prevent cancer development and progression.

# 1. INTRODUCTION

## 1.1. Hyaluronan in tumor progression

Myriad studies attempt to identify specific adhesion molecule(s) expressed in solid tumor cancer (e.g., breast cancer (BC), ovarian cancer, and head and neck squamous cell carcinoma (HNSCC)) that correlate with tumor cell invasive behavior(s). Among such candidate molecules is matrix hyaluronan (HA), a nonsulfated, unbranched glycosaminoglycan consisting of repeating disaccharide units, D-glucuronic acid and *N*-acetyl-D-glucosamine (Lee & Spicer, 2000) which is found in the extracellular matrix (ECM) of most mammalian tissues (Laurent & Fraser, 1992; Toole, 2001). In cancer patients, HA concentrations are usually higher in malignant tumors than in corresponding benign or normal tissues, and in some tumor types, the level of HA is predictive of malignancy. HA is often overexpressed at tumor cell attachment sites and appears to play an important role in promoting tumor cell-specific behaviors (Lee & Spicer, 2000; Toole, 2001; Turley, Nobel, & Bourguignon, 2002). The biosynthesis of HA is regulated by three mammalian HA synthase isozymes: HA synthase1 (HAS1), HA synthase 2 (HAS2), and HA synthase 3 (HAS3) (Itano & Kimata, 1996; Spicer & Nguyen, 1999; Weige, Hascall, & Tammi, 1997; Yamada et al., 1998).

## 1.2. CD44 in tumor progression

CD44 denotes a family of cell surface glycoproteins which are expressed in a variety of cells and tissues including tumor cells and carcinoma tissues (Auvinen, Tammi, Tammi, Johansson, & Kosma, 2005; Bourguignon,

2001; Bourguignon, Gilad, Brightman, Diedrich, & Singleton, 2006; Bourguignon, Gunja-Smith, et al., 1998; Bourguignon, Peyrollier, Gilad, & Brightman, 2007; Bourguignon, Singleton, Diedrich, Stern, & Gilad, 2004; Bourguignon, Singleton, Zhu, & Diedrich, 2003; Bourguignon, Singleton, Zhu, & Zhou, 2002; Bourguignon et al., 1997; Bourguignon, Zhu, Shao, & Chen, 2000; Bourguignon, Zhu, Shao, & Chen, 2001; Bourguignon, Zhu, Shao, Zhu, & Chen, 1999; Bourguignon, Zhu, Zhou, et al., 2001; Bourguignon, Zhu, & Zhu, 1998; Gunthert et al., 1991; Iida & Bourguignon, 1995, 1997; Turley et al., 2002; Zhu & Bourguignon, 1998). Nucleotide sequence analyses reveal that many CD44 isoforms (derived by alternative splicing mechanisms) are variants of the standard form, CD44s isoforms (Screaton et al., 1992). The presence of high levels of CD44 variant (e.g., CD44v3) isoforms is emerging as an important metastatic tumor marker in a number of cancers (Auvinen et al., 2005; Bourguignon, Gunja-Smith, et al., 1998; Bourguignon, Zhu, Shao, et al., 2001; Bourguignon, Zhu, Zhou, et al., 2001; Bourguignon, Zhu, et al., 1998; Bourguignon et al., 1997, 1999, 2000, 2002, 2003; Franzmann, Weed, Civantos, Goodwin, & Bourguignon, 2001; Iida & Bourguignon, 1997; Iida & Bourguignon, 1995; Gunthert et al., 1991; Turley et al., 2002; Zhu & Bourguignon, 1998). Recent studies have shown that CD44 is also expressed in tumor stem cells which have the unique ability to initiate tumor cell-specific properties (Al-Hajj, Wicha, Benito-Hernandez, Morrison, & Clarke, 2003). In fact, CD44 is proposed to be one of the important surface markers for cancer stem cells (CSCs) (Al-Hajj et al., 2003). All CD44 isoforms contain a HA-binding site in their extracellular domain and thereby serve as a major cell surface receptor for HA (Underhill, 1992). Both CD44 isoforms and HA are overexpressed at sites of tumor attachment (Toole & Hascall, 2002). As the histologic grade of each tumor progresses, the percentage of lesions expressing an associated CD44 isoform increases. In particular, the CD44 isoforms are expressed preferentially on highly malignant cancer tissue samples such as BC (Fig. 10.1). In fact, there is a direct correlation between CD44 isoform expression and increased histologic grade of the malignancy in head and neck cancer (Wang & Bourguignon, 2011; Wang, Wong, de Heer, Xia, & Bourguignon, 2009). Several studies indicate that tumor expression of the CD44 isoform may be used as an accurate predictor of overall survival (e.g., nodal status, tumor size, and grade) (Wang & Bourguignon, 2011; Wang, Wong, et al., 2009). It is likely that some of these CD44 isoforms on epithelial cells may act as surface modulators to facilitate unwanted growth factor receptor–growth factor interactions

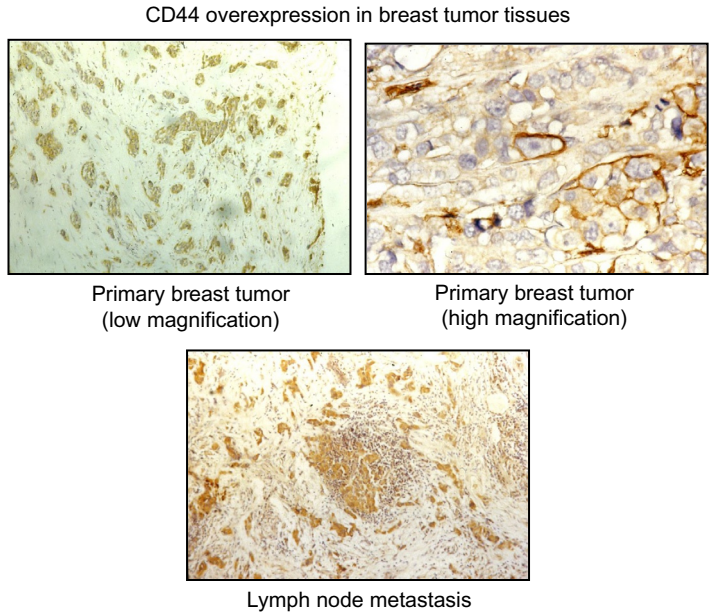


**Figure 10.1** Immunoperoxidase staining of CD44 in human primary breast tumor and lymph node metastasis samples.

(Bourguignon et al., 2006; Wang & Bourguignon, 2006) and subsequent tumor formation. It is also possible that these CD44 isoforms may interact with ECM materials (e.g., HA) such that epithelial cells undergo abnormal mitogenesis and migration. This could be one of the critical steps in tumor invasion and metastasis.

## 2. REGULATION OF TUMOR PROGRESSION BY HA/CD44

The invasive phenotype of tumor cells is linked to cytoskeletal function. Dissection of the transmembrane pathways controlling these cellular processes should aid in understanding the regulatory mechanisms underlying tumor invasion and metastases.

### 2.1. Ankyrin function

CD44 interacts with a number of membrane-associated cytoskeletal proteins, such as ankyrin, which are expressed in a variety of biological systems, including epithelial cells and tissues (Bourguignon, 2001; Bourguignon, Gunja-Smith, et al., 1998; Bourguignon, Zhu, et al., 1998; Turley et al.,

2002; Zhu & Bourguignon, 1998). At the present time, three ankyrin genes have been identified as follows: ankyrin 1 (Ank 1 or ankyrin R), ankyrin 2 (Ank2 or ankyrin B), and ankyrin 3 (Ank3 or ankyrin G), all of which code for ankyrin monomers composed of highly conserved domains and one variable domain (Bennett & Baines, 2001; Lambert et al., 1990). The conserved domain contains a membrane-binding site (~89–95 kDa), also called the ankyrin repeat domain (ARD) in the N-terminal region, and a spectrin-binding domain (~62 kDa). The striking feature shared by all ankyrin N-terminal domain is a repeating 33-amino acid motif present in 24 contiguous copies (Lambert et al., 1990; Lux, John, & Bennett, 1990). The 24 ankyrin repeats within the ARD are known to form binding sites for several distinct membrane protein families, including CD44 (Zhu & Bourguignon, 1998). Overexpression of ARD in cells transfected with ARDcDNA not only interferes with CD44–ankyrin binding but also impairs HA-dependent and CD44-specific biological activities (Lokeshwar, Fregien, & Bourguignon, 1994; Zhu & Bourguignon, 1998). CD44–ankyrin interaction causes cytoskeleton activation and results in several important HA-mediated functions such as cell adhesion, proliferation, and migration (Bourguignon et al., 2007). These observations support the notion that HA-induced CD44–ankyrin interaction is very important for most HA-mediated CD44 functions and tumor-specific behaviors.

## 2.2. RhoGTPase signaling

RhoGTPases (e.g., RhoA, Rac1, and Cdc42) are members of the Rho subclass of the Ras superfamily (Hall, 1998) and are known to cycle between an active GTP-bound state and an inactive GDP-bound state to transmit diverse signals from cell surface receptors to intracellular targets. They function as molecular switches that, in response to external stimuli, regulate key signaling pathways and control a variety of cellular activities (Fig. 10.2) including cytoskeleton reorganization, tumor cell migration, and invasion (Hall, 1998; Li & Lim, 2003). The high incidence of RhoGTPase overexpression in human cancers suggests that these proteins are involved in both cancer onset and progression (Li & Lim, 2003). In fact, overexpression of certain RhoGTPases in human tumors often correlates with a poor prognosis (Fritz, Just, & Kaina, 1999; Suwa et al., 1998). Coordinated RhoGTPase signaling is considered to be a possible mechanism underlying cell proliferation, migration, and invasion, an obvious prerequisite for metastasis (Li & Lim, 2003). HA/CD44-mediated tumor cell-specific

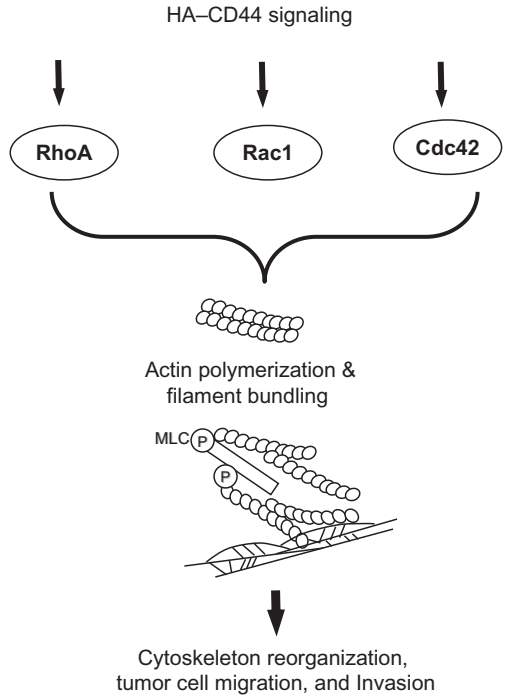


**Figure 10.2** A proposed model for HA/CD44-mediated RhoGTPase signaling and cytoskeleton-mediated functions.

phenotypes are closely linked to the small GTP-binding proteins such as RhoA, Rac1, and Cdc42. Activation of RhoGTPases has been shown to produce specific structural changes in actin assembly, cytoskeleton reorganization, transcriptional activation, tumor cell growth, survival, migration, and invasion (Bourguignon, 2008).

## 2.3. Matrix metalloproteinases activities

It has been suggested that a number of different matrix metalloproteinases (MMPs) (including the 72 kDa gelatinase (gelatinase A, type IV collagenase, MMP-2), the 92 kDa gelatinase (gelatinase B, type V collagenase, MMP-9), the stromelysins (MMP-3, MMP-11), and the interstitial fibroblast-type collagenase (MMP-1)) are thought to play an important role in degrading ECM materials during tumor invasion and metastases (Chen, 1996; Gum et al., 1996). A membrane-type matrix metalloproteinase (MT-MMP, a transmembrane MMP) together with TIMP-2, a tissue inhibitor of metalloproteinase-2 (MMP-2), has been reported to be involved in the

activation of MMP-2 on the cell surface (Polette et al., 1996). Brooks et al. (1996) determined that MMP-2 is localized in a proteolytically active form on the surface of invasive cells based on its ability to bind directly to integrin αvβ3. CD44 isoform (e.g., CD44v3) also found to be closely associated with MMP-9 (gelatinase B) in the plasma membrane of breast tumor cells (Met-1 cell line) (Bourguignon, Gunja-Smith, et al., 1998; Bourguignon, Zhu, et al., 1998). Furthermore, CD44v3-associated MMP-9 appears to be present in a proteolytically active form and preferentially localized at the "invadopodia" structure of the Met-1 cells (Bourguignon, Gunja-Smith, et al., 1998; Bourguignon, Zhu, et al., 1998). Previous findings showed that certain protease(s) is(are) localized on "invadopodia" of human malignant melanoma cells (Chen, 1996). The close interaction between CD44v3 and the active form of MMP-9 in the "invadopodia" structure of Met-1 tumor cells may be required for the degradation of ECM for tumor cell invasion and metastasis. Therefore, we believe that CD44v3 plays an important role in linking ankyrin to the membrane-associated actomyosin contractile system required for upregulating metastatic tumor cell behavior (e.g., invadopodia formation and matrix degradation) and promoting angiogenesis during BC progression.

## 3. ACTIVATION OF CSCS BY HA/CD44

The inexorable progression and therapy-resistant phenotype of cancer cells are driven by uncontrolled growth regulation and adaptive signaling pathways. Despite a trend toward overall improvement in early detection and therapy outcomes, the mortality of solid tumors such as breast cancer (BC) and head and neck cancer (HNSCCs) remains unacceptably high, especially for patients with recurrent or metastatic tumors.

Multidrug resistance is a challenging clinical problem in the treatment of a variety of cancers (Harnett, Kirsten, & Tattersall, 1986). The most commonly used antitumor agents in the treatment of disseminated breast or ovarian cancers include doxorubicin and paclitaxel (taxol) (Gewirtz, 1999). The ability of doxorubicin to bind DNA and/or produce free radicals is thought to be a possible mechanism to induce cytotoxic effects on tumor cells (Gewirtz, 1999). Paclitaxel is known to bind a subunit of the tubulin heterodimers that form cellular microtubules (Bhalla, 2003). The paclitaxel–cytoskeleton interaction accelerates tubulin polymerization and inhibits the depolymerization of microtubules resulting in an inactivation of the mitotic checkpoint and tumor cell killing (Bhalla, 2003). Resistance

to chemotherapeutic drugs used in breast and ovarian cancer treatments (e.g., doxorubicin and paclitaxel) is often associated with overexpression of the multidrug resistance gene 1 (MDR1 or P-glycoprotein (P-gp)). P-gp, the product of the MDR1 (ABCB1) gene, is a transmembrane ATP-dependent transporter molecule known to play a critical role in drug fluxes and chemotherapeutic resistance in a variety of cancers (Baker & El-Osta, 2004; Higgins, 1992). The MDR1 gene product functions as a drug efflux pump actively reducing intracellular drug concentrations in resistant tumor cells (Baker & El-Osta, 2004; Higgins, 1992). Interestingly, recent studies indicate that both HA and CD44 are also involved in chemotherapeutic drug resistance in many cancer types (Bourguignon, Peyrollier, Xia, & Gilad, 2008; Bourguignon et al., 2002; Wang & Bourguignon, 2011). Specifically, HA binding promotes MDR1 expression, cytoskeletal protein (ankyrin)-induced drug fluxes, and chemoresistance in tumor cells (Bourguignon et al., 2008). Furthermore, HA/CD44-mediated ErbB2 signaling and PI3 kinase/AKT-related survival pathways are also involved in chemotherapeutic drug resistance in tumor cells (Misra, Ghatak, Zoltan-Jones, & Toole, 2003).

Accumulating evidence suggests that tumors contain CSCs which contribute at least a part in tumor aggressiveness and resistance to anticancer chemo- and radiotherapy. Accumulating evidence has indicated that most tumors contain a small population of cells which persistently initiate tumor growth and promote tumor progression. These "CSCs" (also called tumor-initiating cells (TICs)) share several hallmarks of normal stem cells (Al-Hajj et al., 2003; Bonnet & Dick, 1997). CSCs are known to undergo self-renewal, maintain quiescence, display multipotency, and express survival protein/antiapoptosis proteins (Al-Hajj et al., 2003; Bonnet & Dick, 1997; Bourguignon, Earle, Wong, Spevak, & Krueger, 2012; Bourguignon, Wong, Earle, & Chen, 2012). Another well-known property of CSCs is their ability to expand the stem cell population by undergoing cell proliferation/survival and/or clone formation and differentiation (Al-Hajj et al., 2003; Bonnet & Dick, 1997; Bourguignon, Earle, et al., 2012; Bourguignon, Wong, et al., 2012). These CSCs have been shown both experimentally and clinically to be resistant to radiation and chemotherapy potentially resulting in residual disease that leads to recurrence. A number of studies have identified specific molecules expressed in CSCs that correlate with both stem cell properties and tumor cell behaviors. CD44 is expressed in both normal stem cells and CSCs and serves as an important stem cell marker (Al-Hajj et al., 2003). A number of studies now indicate that tumor

cells with high levels of CD44 expression appear to exhibit CSC properties in many cancers (Bourguignon, Earle, et al., 2012; Bourguignon, Wong, et al., 2012).

### 3.1. CD44v3, a newly identified CSC marker

All stem cells are thought to exist in specialized microenvironments known as niches (Astachov, Vago, Aviv, & Nevo, 2011; Haylock & Nilsson, 2006). Components present in the niches can regulate stem cell behavior through direct binding to stem cell surface receptors or via indirect activation of paracrine signaling (Astachov et al., 2011; Haylock & Nilsson, 2006). ECM components including HA are known to be present in one of the stem cell niches (Astachov et al., 2011; Haylock & Nilsson, 2006). HA–CD44 interaction enhances sphere formation (cell–cell adhesion) (Fig. 10.3A), self-renewal/growth, and clonal formation in highly tumorigenic CD44v3$^{high}$ALDH1$^{high}$ tumor cell population (Bourguignon, Wong, et al.,, 2012). These observations support the contention that HA signaling is directly involved in the regulation of CSC-like properties in CD44v3$^{high}$ALDH1$^{high}$ cells.

CD44 is commonly expressed in various isoforms generated by alternative mRNA splicing of variant exons inserted into an extracellular membrane-proximal site (Screaton et al., 1992). CD44 is expressed in both

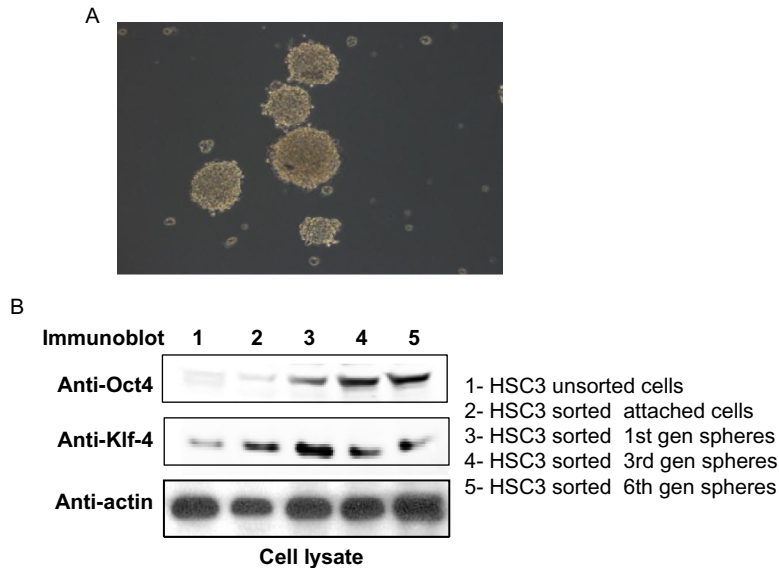


**Figure 10.3** HA/CD44-mediated sphere formation (A) and stem cell marker expression in sphere cells with multiple generations of passages (B).

normal and CSCs and serves as an important stem cell marker (Al-Hajj et al., 2003). It is now known that neither CD44s nor CD44v6 distinguishes normal from benign or malignant epithelia of head and neck (Mack & Gires, 2008). Consequently, the identification of other specific CD44 isoform-related CSC markers is needed. In this regard, the role of CD44v3 isoform in head and neck cancer progression has been highlighted in studies that have identified an association of the v3-containing isoform with head and neck cancer growth, migration, and MMP expression (Wang & Bourguignon, 2011; Wang, Peyrollier, & Bourguignon, 2007; Wang, Wreesmann, & Bourguignon, 2007). Transfection of the CD44v3 isoform into a non-expressing head and neck cancer cell line results in significantly increased tumor cell migration (Wang, Peyrollier, et al., 2007; Wang, Wreesmann, et al., 2007). In addition, treatment of a CD44v3 isoform-expressing head and neck cancer cell line with anti-CD44v3 isoform antibody decreased *in vitro* proliferation and increased cisplatin sensitivity (Wang, Peyrollier, et al., 2007; Wang, Wreesmann, et al., 2007). Using the same anti-CD44v3 isoform antibody, immunohistochemical tissue analysis revealed that the CD44v3 isoform is preferentially expressed in metastatic lymph nodes. Furthermore, CD44v3 isoform expression in primary tumors is significantly associated with advanced T status and positive lymph nodes (Wang & Bourguignon, 2011; Wang, Peyrollier, et al., 2007; Wang, Wong, et al., 2009; Wang, Wreesmann, et al., 2007). These findings suggest that the CD44v3 isoform is closely associated with head and neck cancer development and progression and may serve as a new CSC marker.

Recently, an elevated level of aldehyde dehydrogenase 1 (ALDH1) activity was also shown to identify CSCs in head and neck cancer (Bourguignon, Wong, et al., 2012). The subpopulation of $CD44v3^{high}ALDH1^{high}$ cells expressing stem cell markers such as Oct4 and Klf4 (isolated from head and neck tumor cells) appears to undergo cell growth and self-renewing after multiple passages from spheres (demonstrated by serial passage of sphere-forming ability and long-term tumor cell growth) (Fig. 10.3B). Importantly, these $CD44v3^{high}ALDH1^{high}$ cells exhibit CSC-like phenotypes and are highly tumorigenic for generating tumors starting with as few as 50 cells. In breast cancer, a $CD44^{high}CD24^{low}$ tumor population has also been shown to be highly tumorigenic (Bourguignon, Earle, et al., 2012; Bourguignon, Wong, et al., 2012).

One of the characteristics of CSCs is overexpression of stem cell-specific transcription factors such as Oct4, Sox2, and Nanog (Boyer et al., 2005;

Kashyap et al., 2009). Previous studies indicate that Oct4, Sox2, and Nanog often function in combinatorial complexes to regulate the expression of gene loci which are involved in self-renewal, proliferation, and differentiation (Boyer et al., 2005; Kashyap et al., 2009). These three molecules are also closely associated with higher histological grades and poorer clinical survival in head and neck cancer (Bourguignon, Earle, et al., 2012; Dong, Wilhelm, & Koopman, 2004; Wang, Wong, et al., 2009). One of the characteristics of CSCs is overexpression of stem cell-specific transcription factors such as Oct4, Sox2, and Nanog (Boyer et al., 2005). These three molecules are also closely associated with higher histological grades and poorer clinical survival in head and neck cancer (Bourguignon, Earle, et al., 2012; Dong et al., 2004; Wang, Wong, et al., 2009). Moreover, a high level of Oct4, Sox2, and Nanog expression is detected in $CD44v3^{high}ALDH1^{high}$ cell-induced mouse tumors and in human head and neck cancer patient specimens (Bourguignon, Wong, et al., 2012). These findings indicate that $CD44v3^{high}ALDH1^{high}$ cells are likely to be generating tumors through the stem cell processes of self-renewal, growth, and differentiation. These cells can persist in tumors as a distinct population, possibly causing chemotherapy resistance and tumor recurrence at a later time. The mechanism by which the regulatory network consisting of Oct4, Sox2, and Nanog contributes to the establishment of CSC-specific phenotypes in human cancer is described below.

## 3.2. Nanog/Oct4/Sox2 & miR-302-regulated CSC functions

Previous genetic studies using mouse models revealed that transcription factors such as Oct4, Sox2, and Nanog have distinct roles but could use similar signaling pathways to maintain "stemness" functions during development (Boyer et al., 2005; Kashyap et al., 2009). Some studies have also indicated that Oct4, Sox2, and Nanog cooccupy the promoter of miR-302 which has been shown to target genes required for development and oncogenesis (Card et al., 2008; Liu et al., 2011). At the transcriptional level, Oct4, Sox2, and Nanog form a positive autoregulatory loop that is important for the maintenance of the undifferentiated state. At the post-translational level, miR-302 family is emerging as a key player in the control of cell proliferation and cell fate determination during differentiation (Card et al., 2008; Liu et al., 2011). Recently, new evidence indicates that the HA–CD44v3 interaction activates Oct4, Sox2, and Nanog complex

formation in CD44v3-expressing CSCs. Furthermore, our results indicate that the miR-302 cluster is controlled by a promoter containing Oct4/Sox2/Nanog-binding sites in these cells. The production of miR-302 is Oct4/Sox2/Nanog dependent in CSCs. Overexpression of miR-302a and miR-302b in both mouse tumors induced by CD44v3-expressing CSC cells can be detected in human head and neck cancer patient samples (Bourguignon, Wong, et al., 2012). These findings clearly established the existence of a close association between miR-302 clusters (e.g., miR-302a and miR-302b) and head and neck cancer development.

Inhibitors of the apoptosis family of proteins (IAPs, e.g., cIAP-1, cIAP-2, and XIAP) are also frequently overexpressed by CSCs. Importantly, upregulation of IAPs increases cell survival by the binding of IAPs to caspases and suppressing apoptosis (Hunter, LaCasse, & Korneluk, 2007). A recent study indicated that HA enhances antiapoptotic effects and decreases the ability of cisplatin to induce apoptosis and cell death of these $CD44v3^{high}ALDH1^{high}$ cells leading to the enhancement of chemoresistance (Bourguignon, Wong, et al., 2012). These findings suggest that HA-mediated cell survival contributes to both a decrease in apoptosis/cell death and an increase in cisplatin resistance in these CSC-like $CD44v3^{high}ALDH1^{high}$ cells. Furthermore, downregulation of miR-302a or miR-302b by transfecting $CD44v3^{high}ALDH1^{high}$ cells with either anti-miR-302a or miR-302b inhibitor effectively reduces HA-mediated IAP protein (e.g., cIAP-1, cIAP-2, and XIAP) expression in $CD44v3^{high}ALDH1^{high}$ cells. Consequently, the level of cisplatin-induced apoptosis and cell death of these cells is also significantly elevated. Taken together, we believe that the miR-302 cluster (e.g., miR-302a and miR-302b) plays a critical role in regulating HA/CD44-mediated chemotherapy resistance in these tumorigenic and CSC-like $CD44v3^{high}ALDH1^{high}$ cell populations from head and neck cancer. Several years ago, a small molecule IAP protein inhibitor (bivalent SM-164) was shown to concurrently target cIAP-1/cIAP-2 for degradation and antagonize XIAP leading to an enhancement of tumor cell apoptosis and tumor regression (Lu et al., 2008). Recently, we reported that chemosensitivity in CSC-like $CD44v3^{high}ALDH1^{high}$ cells increased with the newly developed IAP protein inhibitor, SM164 (Bourguignon, Wong, et al., 2012). These findings suggest that the use of a combination of IAP protein inhibitor (SM164) and cisplatin may significantly improve the efficacy of chemotherapeutic drug treatment by targeting CSCs in head and neck cancer.

### 3.3. Nanog & miR-21-regulated CSC functions

Cisplatin is one of the most common antihead and neck cancer chemotherapy drugs used today. Various mechanisms of cisplatin resistance in head and neck cancer have been proposed with potential molecular effectors of resistance, including p53, glutathione *S*-transferase, and ErbB2 (Bradford et al., 2003; Kato et al., 2000; Shiga et al., 2000). The ability of this drug to induce tumor cell death is also counteracted by the presence of antiapoptotic regulators leading to chemoresistance (Wang & Bourguignon, 2011). Activation of several other HA–CD44-mediated oncogenic signaling pathways (e.g., intracellular $Ca^{2+}$ mobilization (Wang & Bourguignon, 2006; Bourguignon et al., 2006), epidermal growth factor receptor-mediated ERK signaling (Bourguignon et al., 2006; Wang & Bourguignon, 2006), and topoisomerase activation (Wang, Peyrollier, et al., 2007; Wang, Wreesmann, et al., 2007)) leads to multidrug resistance in a number of tumor cells. However, the mechanism by which HA/CD44 activates oncogenesis and drug resistance in HNSCC has not been fully elucidated.

The functional significance of miR-21 has been elucidated in several recent studies following the discovery of its specific targets (Asangani et al., 2008), making miR-21 one of the most-studied miRNAs due to its involvement in cancer progression. Importantly, miR-21 plays a role in the inhibition of tumor suppressor proteins such as program cell death 4 (PDCD4), via a conserved site within the 3′-UTR (3′-untranslated region) of the mRNA (Asangani et al., 2008; Bourguignon, Spevak, Wong, Xia, & Gilad, 2009). Downregulation of PDCD4 expression by miR-21 leads to tumor cell growth, survival, chemoresistance, invasion, and metastasis (Bourguignon et al., 2009). Thus, miR-21 is currently considered to be an oncogenic miRNA. In previous studies, a new matrix HA/CD44-mediated Nanog/Stat-3 signaling mechanism was reported to be involved in miR-21 production and chemotherapy chemoresistance in breast tumor cells and HNSCC cells (Bourguignon, Earle, et al., 2012). Specifically, these results indicated that HA/CD44 activates Nanog–Stat-3 signaling which, in turn, stimulates miR-21 expression and function. These events lead to the reduction of the tumor suppressor protein, PDCD4, upregulation of survival proteins, IAP family (e.g., cIAP-1, cIAP-2 and XIAP), and cisplatin chemoresistance in HNSCC cells (Bourguignon, Earle, et al., 2012). Inhibition of either Nanog/Stat-3 signaling or silencing miR-21 expression/function results in PDCD4 upregulation and causes a reduction of survival protein expression and an enhancement of

chemosensitivity to cisplatin (Bourguignon, Earle, et al., 2012). Thus, these findings strongly support the contention that Nanog, Stat-3, and miR-21 form a functional signaling axis that regulates tumor cell survival and cisplatin chemoresistance in tumor cells.

### 3.4. ErbB2, p53, and CD44 signaling in CSCs

The p53 tumor suppressor is a key mediator of cellular responses to various stresses. A previous study showed that p53 inhibits CD44 expression via binding to a noncanonical p53-binding sequence in the CD44 promoter (Godar et al., 2008). This interaction enables an untransformed cell to respond to stress-induced, p53-dependent cytostatic and apoptotic signals that would otherwise be blocked by the actions of CD44. In the absence of p53 function, the resulting derepressed CD44 expression is essential for the growth and tumor-initiating ability of highly tumorigenic mammary epithelial cells. Thus, CD44 appears to function as a key tumor-promoting agent in transformed tumor cells lacking p53 function.

CSCs from breast cancer cells are able to form colonies or new tumors (Al-Hajj et al., 2003; Bonnet & Dick, 1997). Specific cell surface markers such as $CD44^{high}/CD24^{-/low}$ in breast CSCs (Al-Hajj et al., 2003) are shown to have increased expression of proinvasive genes required for metastasis such as IL-1α, IL-6, IL-8, and urokinase plasminogen activator (Sheridan et al., 2006). Additionally, CSCs appear to exhibit notable chemo- and radioresistance. For example, in tumor cells, under genotoxic stress conditions (such as ionizing radiation), activation of prosurvival pathways and inhibition of proapoptotic pathways are responsible for tumor radioresistance. The molecules involved in these pathways are potential targets to enhance tumor radiosensitivity. Clinical data suggest that breast cancer patients with tumors overexpressing ErbB2/HER2/neu, a member of ErbB family of receptor tyrosine kinases, show a poor prognosis compared to ErbB2/HER2/neu-negative tumors, and the ErbB2/HER2 gene enhancement is correlated with the time to relapse of the disease (Slamon et al., 1987). The poorer prognosis of women with ErbB2/HER2/Neu-amplified tumors is entirely abrogated if they are treated with anti-ErbB2/HER2 therapy. Upon irradiation, cells overexpressing ErbB2/HER2 are able to activate NFκB, a key transcription factor in stress response that regulates a prosurvival network (Guo et al., 2004). Importantly, the NFκB-binding motif in the ErbB2/HER2 gene promoter region is found to be responsible for radiation-induced HER2 expression (Cao et al., 2009),

and a recent study indicated that HER2-positive CSCs are present in "ErbB2/HER2-negative" breast cancer cells and the HER2-positive CSCs are increased in the recurrent/metastatic tumors (Duru et al., 2012), indicating that ErbB2/HER2 is a dynamic feature of CSCs in breast cancer. Defining the central role of ErbB2/HER2-positive CSCs may offer effective targets for breast cancer treatment. These findings suggest that the existence of different subpopulations of CSCs with varied sets of mutations and genomic alterations may be likely since heterogeneous tumors consist of unstable genomes and different responses to anticancer therapy. During chemo- and/or radiotherapy, the most resistant CSCs would be selected and continue to sustain the tumor under treatments. These findings shed light onto a new conceptual paradigm of how CSCs or TICs contribute to the radiation response. Identification of CSC-associated radioresistance needs to be further evaluated in clinical studies.

## 4. CONCLUSION

HA/CD44-mediated tumor cell-specific phenotype is closely linked to cytoskeletal functions which involve membrane-associated cytoskeletal proteins (e.g., ankyrin and GTPases) and MMPs. Activation of ankyrin, GTPases, and MMPs produces specific structural changes in actin assembly, cytoskeleton reorganization, transcriptional activation, tumor cell growth, survival, migration, and invasion. These events generate coordinated "cross talk" among multiple signaling pathways required for oncogenesis. Accumulating evidence also indicates that Oct4/Sox2/Nanog signaling and microRNA (e.g., miR-302 and miR-21) expression occur in HA/CD44-activated CSCs. Furthermore, CD44-expressing CSCs require downregulation of p53 and upregulation of ErbB2. All these events contribute to CSC self-renewal, clonal formation, antiapoptosis/survival, radiation resistance, and chemoresistance leading to tumor progression. A current model illustrating HA-dependent and CD44-specific signaling pathways is described in Fig. 10.4.

## ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Dr. Gerard J. Bourguignon in the preparation and review of this manuscript. This work was supported by Veterans Affairs (VA) Merit Review Awards (RR & D-1I01 RX000601 and BLR & D-5I01 BX000628), United States Public Health Grants (R01 CA66163). L. Y. W. B is a VA Senior Research Career Scientist.

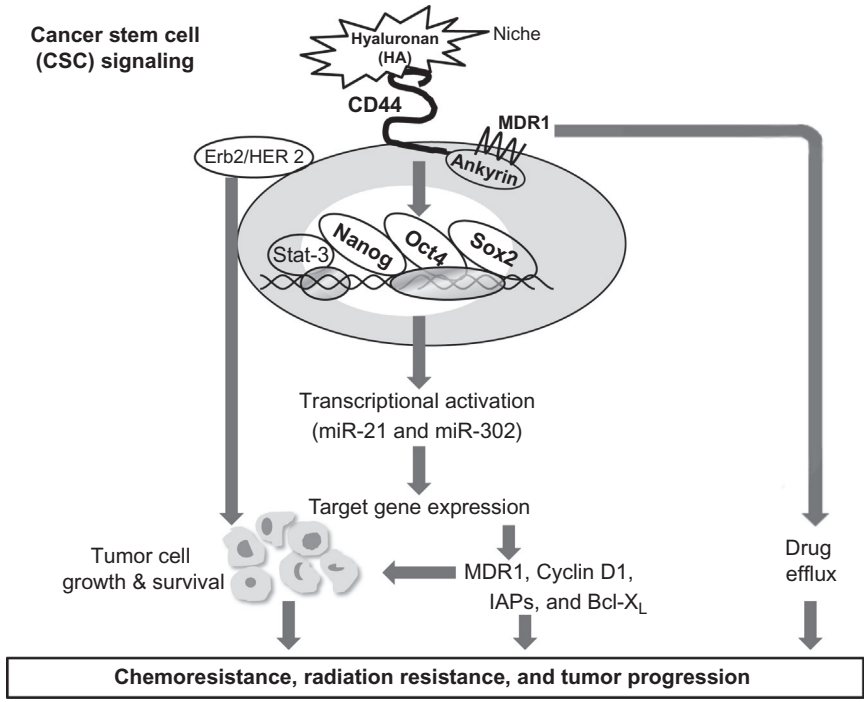


**Figure 10.4** A proposed model for cancer stem cell signaling and tumor progression.

## REFERENCES

Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J., & Clarke, M. F. (2003). Prospective identification of tumorigenic breast cancer cells. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 3983–3988.

Asangani, I. A., Rasheed, S. A., Nikolova, D. A., Leupold, J. H., Colburn, N. H., Post, S., et al. (2008). MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene*, *27*, 2128–2136.

Astachov, L., Vago, R., Aviv, M., & Nevo, Z. (2011). Hyaluronan and mesenchymal stem cells: From germ layer to cartilage and bone. *Frontiers in Bioscience (Landmark Edition)*, *16*, 261–276.

Auvinen, P., Tammi, R., Tammi, M., Johansson, R., & Kosma, V. M. (2005). Expression of CD44s, CD44v3 and CD44v6 in benign and malignant breast lesions: Correlation and colocalization with hyaluronan. *Histopathology*, *47*, 420–428.

Baker, E. K., & El-Osta, A. (2004). MDR1, chemotherapy and chromatin remodeling. *Cancer Biology & Therapy*, *3*, 819–824.

Bennett, V., & Baines, A. J. (2001). Spectrin and ankyrin-based pathways: Metazoan inventions for integrating cells into tissues. *Physiological Reviews*, *81*, 1353–1392.

Bhalla, K. N. (2003). Microtubule-targeted anticancer agents and apoptosis. *Oncogene*, *22*, 9075–9086.

Bonnet, D., & Dick, J. E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nature Medicine*, *3*, 730–737.

Bourguignon, L. Y. (2001). CD44-mediated oncogenic signaling and cytoskeleton activation during mammary tumor progression. *Journal of Mammary Gland Biology and Neoplasia*, *6*, 287–297.

Bourguignon, L. Y. (2008). Hyaluronan-mediated CD44 activation of RhoGTPase signaling and cytoskeleton function promotes tumor progression. *Seminars in Cancer Biology*, *18*, 251–259.

Bourguignon, L. Y., Earle, C., Wong, G., Spevak, C. C., & Krueger, K. (2012). Stem cell marker (Nanog) and Stat-3 signaling promote MicroRNA-21 expression and chemoresistance in hyaluronan/CD44-activated head and neck squamous cell carcinoma cells. *Oncogene*, *31*, 149–160.

Bourguignon, L. Y., Gilad, E., Brightman, A., Diedrich, F., & Singleton, P. (2006). Hyaluronan-CD44 interaction with leukemia-associated RhoGEF and epidermal growth factor receptor promotes Rho/Ras co-activation, phospholipase C epsilon-Ca2+ signaling, and cytoskeleton modification in head and neck squamous cell carcinoma cells. *The Journal of Biological Chemistry*, *281*, 14026–14040.

Bourguignon, L. Y., Gunja-Smith, Z., Iida, N., Zhu, H. B., Young, L. J., Muller, W. J., et al. (1998). CD44v(3,8-10) is involved in cytoskeleton-mediated tumor cell migration and matrix metalloproteinase (MMP-9) association in metastatic breast cancer cells. *Journal of Cellular Physiology*, *176*, 206–215.

Bourguignon, L. Y., Peyrollier, K., Gilad, E., & Brightman, A. (2007). Hyaluronan-CD44 interaction with neural Wiskott-Aldrich syndrome protein (N-WASP) promotes actin polymerization and ErbB2 activation leading to beta-catenin nuclear translocation, transcriptional up-regulation, and cell migration in ovarian tumor cells. *The Journal of Biological Chemistry*, *282*, 1265–1280.

Bourguignon, L. Y., Peyrollier, K., Xia, W., & Gilad, E. (2008). Hyaluronan-CD44 interaction activates stem cell marker Nanog, Stat-3-mediated MDR1 gene expression, and ankyrin-regulated multidrug efflux in breast and ovarian tumor cells. *The Journal of Biological Chemistry*, *283*, 17635–17651.

Bourguignon, L. Y., Singleton, P. A., Diedrich, F., Stern, R., & Gilad, E. (2004). CD44 interaction with Na+-H+ exchanger (NHE1) creates acidic microenvironments leading to hyaluronidase-2 and cathepsin B activation and breast tumor cell invasion. *The Journal of Biological Chemistry*, *279*, 26991–27007.

Bourguignon, L. Y., Singleton, P. A., Zhu, H., & Diedrich, F. (2003). Hyaluronan-mediated CD44 interaction with RhoGEF and Rho kinase promotes Grb2-associated binder-1 phosphorylation and phosphatidylinositol 3-kinase signaling leading to cytokine (macrophage-colony stimulating factor) production and breast tumor progression. *The Journal of Biological Chemistry*, *278*, 29420–29434.

Bourguignon, L. Y., Singleton, P. A., Zhu, H., & Zhou, B. (2002). Hyaluronan promotes signaling interaction between CD44 and the transforming growth factor beta receptor I in metastatic breast tumor cells. *The Journal of Biological Chemistry*, *277*, 39703–39710.

Bourguignon, L. Y., Spevak, C. C., Wong, G., Xia, W., & Gilad, E. (2009). Hyaluronan-CD44 interaction with protein kinase C(epsilon) promotes oncogenic signaling by the stem cell marker Nanog and the production of microRNA-21, leading to down-regulation of the tumor suppressor protein PDCD4, anti-apoptosis, and chemotherapy resistance in breast tumor cells. *The Journal of Biological Chemistry*, *284*, 26533–26546.

Bourguignon, L. Y., Wong, G., Earle, C., & Chen, L. (2012). Hyaluronan-CD44v3 interaction with Oct4-Sox2-Nanog promotes miR-302 expression leading to self-renewal, clonal formation, and cisplatin resistance in cancer stem cells from head and neck squamous cell carcinoma. *The Journal of Biological Chemistry*, *287*, 32800–32824.

Bourguignon, L. Y., Zhu, H., Chu, A., Iida, N., Zhang, L., & Hung, M. C. (1997). Interaction between the adhesion receptor, CD44, and the oncogene product, p185HER2,

promotes human ovarian tumor cell activation. *The Journal of Biological Chemistry*, *272*, 27913–27918.
Bourguignon, L. Y., Zhu, H., Shao, L., & Chen, Y. W. (2000). CD44 interaction with tiam1 promotes Rac1 signaling and hyaluronic acid-mediated breast tumor cell migration. *The Journal of Biological Chemistry*, *275*, 1829–1838.
Bourguignon, L. Y., Zhu, H., Shao, L., & Chen, Y. W. (2001). CD44 interaction with c-Src kinase promotes cortactin-mediated cytoskeleton function and hyaluronic acid-dependent ovarian tumor cell migration. *The Journal of Biological Chemistry*, *276*, 7327–7336.
Bourguignon, L. Y., Zhu, H., Shao, L., Zhu, D., & Chen, Y. W. (1999). Rho-kinase (ROK) promotes CD44v(3,8-10)-ankyrin interaction and tumor cell migration in metastatic breast cancer cells. *Cell Motility and the Cytoskeleton*, *43*, 269–287.
Bourguignon, L. Y., Zhu, H., Zhou, B., Diedrich, F., Singleton, P. A., & Hung, M. C. (2001). Hyaluronan promotes CD44v3-Vav2 interaction with Grb2-p185(HER2) and induces Rac1 and Ras signaling during ovarian tumor cell migration and growth. *The Journal of Biological Chemistry*, *276*, 48679–48692.
Bourguignon, L. Y., Zhu, D., & Zhu, H. (1998). CD44 isoform-cytoskeleton interaction in oncogenic signaling and tumor progression. *Frontiers in Bioscience*, *3*, d637–d649.
Boyer, L. A., Lee, T. I., Cole, M. F., Johnstone, S. E., Levine, S. S., Zucker, J. P., et al. (2005). Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell*, *122*, 947–956.
Bradford, C. R., Zhu, S., Ogawa, H., Ogawa, T., Ubell, M., Narayan, A., et al. (2003). P53 mutation correlates with cisplatin sensitivity in head and neck squamous cell carcinoma lines. *Head & Neck*, *25*, 654–661.
Brooks, P. C., Strömblad, S., Sanders, L. C., von Schalscha, T. L., Aimes, R. T., Stetler-Stevenson, W. G., et al. (1996). Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. *Cell*, *85*, 683–693.
Cao, N., Li, S., Wang, Z., Ahmed, K. M., Degnan, M. E., Fan, M., et al. (2009). NF-kappaB-mediated HER2 overexpression in radiation-adaptive resistance. *Radiation Research*, *171*, 9–21.
Card, D. A., Hebbar, P. B., Li, L., Trotter, K. W., Komatsu, Y., Mishina, Y., et al. (2008). Oct4/Sox2-regulated miR-302 targets cyclin D1 in human embryonic stem cells. *Molecular and Cellular Biology*, *28*, 6426–6438.
Chen, W. T. (1996). Proteases associated with invadopodia, and their role in degradation of extracellular matrix. *Enzyme & Protein*, *49*, 59–71.
Dong, C., Wilhelm, D., & Koopman, P. (2004). Sox genes and cancer. *Genome Research*, *105*, 442–447.
Duru, N., Fan, M., Candas, D., Menaa, C., Liu, H. C., Nantajit, D., et al. (2012). HER2-associated radioresistance of breast cancer stem cells isolated from HER2-negative breast cancer cells. *Clinical Cancer Research*, *18*, 6634–6647.
Franzmann, E. J., Weed, D. T., Civantos, F. J., Goodwin, W. J., & Bourguignon, L. Y. (2001). A novel CD44 v3 isoform is involved in head and neck squamous cell carcinoma progression. *Otolaryngology and Head and Neck Surgery*, *124*, 426–432.
Fritz, G., Just, I., & Kaina, B. (1999). RhoGTPase over-expressed in human tumors. *International Journal of Cancer*, *81*, 682–687.
Gewirtz, D. A. (1999). A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochemical Pharmacology*, *57*, 727–741.
Godar, S., Ince, T. A., Bell, G. W., Feldser, D., Donaher, J. L., Bergh, J., et al. (2008). Growth-inhibitory and tumor- suppressive functions of p53 depend on its repression of CD44 expression. *Cell*, *134*, 62–73.

Gum, R., Lengyel, E., Juarez, J., Chen, J. H., Sato, H., Seiki, M., et al. (1996). Stimulation of 92-kDa gelatinase B promoter activity by ras is mitogen-activated protein kinase kinase 1-independent and requires multiple transcription factor binding sites including closely spaced PEA3/ets and AP-1 sequences. *The Journal of Biological Chemistry*, *271*, 10672–10680.

Gunthert, U., Hoffman, M., Rudy, W., Reber, S., Zoller, M., Haußmann, I., et al. (1991). A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell*, *65*, 13–24.

Guo, G., Wang, T., Gao, Q., Tamae, D., Wong, P., Chen, T., et al. (2004). Expression of ErbB2 enhances radiation-induced NF-kappaB activation. *Oncogene*, *23*, 535–545.

Hall, A. (1998). Rho GTPase and the actin cytoskeleton. *Science*, *279*, 509–514.

Harnett, P. R., Kirsten, F., & Tattersall, M. H. (1986). Drug resistance in clinical practice: Patterns of treatment failure in advanced breast and ovarian cancer. *Journal of Clinical Oncology*, *4*, 952–957.

Haylock, D. N., & Nilsson, S. K. (2006). The role of hyaluronic acid in hemopoietic stem cell biology. *Regenerative Medicine*, *1*, 437–445.

Higgins, C. F. (1992). ABC transporters: From microorganisms to man. *Annual Review of Cell Biology*, *8*, 67–113.

Hunter, A. M., LaCasse, E. C., & Korneluk, R. G. (2007). The inhibitors of apoptosis (IAPs) as cancer targets. *Apoptosis*, *12*, 1543–1568.

Iida, N., & Bourguignon, L. Y. (1995). New CD44 splice variants associated with human breast cancers. *Journal of Cellular Physiology*, *162*, 127–133.

Iida, N., & Bourguignon, L. Y. (1997). Coexpression of CD44 variant (v10/ex14) and CD44S in human mammary epithelial cells promotes tumorigenesis. *Journal of Cellular Physiology*, *171*, 152–160.

Itano, N., & Kimata, K. (1996). Expression cloning and molecular characterization of HAS protein, a eukaryotic hyaluronan synthase. *The Journal of Biological Chemistry*, *271*, 9875–9878.

Kashyap, V., Rezende, N. C., Scotland, K. B., Shaffer, S. M., Persson, J. L., Gudas, L. J., et al. (2009). Regulation of stem cell pluripotency and differentiation involves a mutual regulatory circuit of the NANOG, OCT4, and SOX2 pluripotency transcription factors with polycomb repressive complexes and stem cell microRNAs. *Stem Cells and Development*, *18*, 1093–1108.

Kato, T., Duffey, D. C., Ondrey, F. G., Dong, G., Chen, Z., Cook, J. A., et al. (2000). Cisplatin and radiation sensitivity in human head and neck squamous carcinomas are independently modulated by glutathione and transcription factor NF-kappaB. *Head & Neck*, *22*, 748–759.

Lambert, S., Yu, H., Prchal, J. T., Lawler, J., Ruff, P., Speicher, D., et al. (1990). cDNA sequence for human erythrocyte ankyrin. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 1730–1734.

Laurent, T. C., & Fraser, J. R. E. (1992). Hyaluronan. *FASEB Journal*, *6*, 2397–2404.

Lee, J. Y., & Spicer, A. P. (2000). Hyaluronan: A multifunctional, megadalton, stealth molecule. *Current Opinion in Cell Biology*, *12*, 581–586.

Li, X., & Lim, B. (2003). RhoGTPases and their role in cancer. *Oncology Research*, *13*, 323–331.

Liu, H., Deng, S., Zhao, Z., Zhang, H., Xiao, J., Song, W., et al. (2011). Oct4 regulates the miR-302 cluster in P19 mouse embryonic carcinoma cells. *Molecular Biology Reports*, *38*, 2155–2160.

Lokeshwar, V. B., Fregien, N., & Bourguignon, L. Y. (1994). Ankyrin-binding domain of CD44(GP85) is required for the expression of hyaluronic acid-mediated adhesion function. *The Journal of Cell Biology*, *126*, 1099–1109.

Lu, J., Bai, L., Sun, H., Nikolovska-Coleska, Z., McEachern, D., Qiu, S., et al. (2008). SM-164: A novel, bivalent Smac mimetic that induces apoptosis and tumor regression by concurrent removal of the blockade of cIAP-1/2 and XIAP. *Cancer Research*, *68*, 9384–9393.

Lux, S. E., John, K. M., & Bennett, V. (1990). Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins. *Nature*, *344*, 36–42.

Mack, B., & Gires, O. (2008). CD44s and CD44v6 expression in head and neck epithelia. *PLoS One*, *3*, e3360.

Misra, S., Ghatak, S., Zoltan-Jones, A., & Toole, B. P. (2003). Regulation of multidrug resistance in cancer cells by hyaluronan. *The Journal of Biological Chemistry*, *278*, 25285–25288.

Polette, M., Nawrocki, B., Gilles, C., Sato, H., Seiki, M., Tournier, J. M., et al. (1996). MT-MMP expression and localisation in human lung and breast cancers. *Virchows Archiv*, *428*, 29–35.

Screaton, G. R., Bell, M. V., Jackson, D. G., Cornelis, F. B., Gerth, U., & Bell, J. I. (1992). Genomic structure of DNA coding the lymphocyte homing receptor CD44 reveals 12 alternatively spliced exons. *Proceedings of the National Academy of Sciences of the United States of America*, *89*, 12160–12164.

Sheridan, C., Kishimoto, H., Fuchs, R. K., Mehrotra, S., Bhat-Nakshatri, P., Turner, C. H., et al. (2006). CD44+/CD24- breast cancer cells exhibit enhanced invasive properties: An early step necessary for metastasis. *Breast Cancer Research*, *8*, R59.

Shiga, H., Rasmussen, A. A., Johnston, P. G., Langmacher, M., Baylor, A., Lee, M., et al. (2000). Prognostic value of c-erbB2 and other markers in patients treated with chemotherapy for recurrent head and neck cancer. *Head & Neck*, *22*, 599–608.

Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., & McGuire, W. L. (1987). Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*, *235*, 177–182.

Spicer, A. P., & Nguyen, T. K. (1999). Mammalian hyaluronan synthases: Investigation of functional relationships in vivo. *Biochemical Society Transactions*, *27*, 109–115.

Suwa, H., Ohshio, G., Imamura, T., Watanabe, G., Arii, S., Imamura, M., et al. (1998). Overexpression of the rho C gene correlates with progression of ductal adenocarcinoma of the pancreas. *British Journal of Cancer*, *77*, 147–152.

Toole, B. P. (2001). Hyaluronan in morphogenesis. *Seminars in Cell & Developmental Biology*, *12*, 79–87.

Toole, B. P., & Hascall, V. C. (2002). Hyaluronan and tumor growth. *The American Journal of Pathology*, *161*, 745–747.

Turley, E. A., Nobel, P. W., & Bourguignon, L. Y. W. (2002). Signaling properties of hyaluronan receptors. *The Journal of Biological Chemistry*, *277*, 4589–4592.

Underhill, C. (1992). CD44: The hyaluronan receptor. *Journal of Cell Science*, *103*, 293–298.

Wang, S. J., & Bourguignon, L. Y. (2006). Hyaluronan and the interaction between CD44 and epidermal growth factor receptor in oncogenic signaling and chemotherapy resistance in head and neck cancer. *Archives of Otolaryngology—Head & Neck Surgery*, *132*, 771–778.

Wang, S. J., & Bourguignon, L. Y. (2011). Role of hyaluronan-mediated CD44 signaling in head and neck squamous cell carcinoma progression and chemoresistance. *The American Journal of Pathology*, *178*, 956–963.

Wang, S. J., Peyrollier, K., & Bourguignon, L. Y. (2007). The influence of hyaluronan-CD44 interaction on topoisomerase II activity and etoposide cytotoxicity in head and neck cancer. *Archives of Otolaryngology—Head & Neck Surgery*, *133*, 281–288.

Wang, S. J., Wong, G., de Heer, A. M., Xia, W., & Bourguignon, L. Y. (2009). CD44 variant isoforms in head and neck squamous cell carcinoma progression. *Laryngoscope*, *119*, 1518–1530.

Wang, S. J., Wreesmann, V. B., & Bourguignon, L. Y. (2007). Association of CD44 V3-containing isoforms with tumor cell growth, migration, matrix metalloproteinase expression, and lymph node metastasis in head and neck cancer. *Head & Neck*, *29*, 550–558.

Weige, P. H., Hascall, V. C., & Tammi, M. (1997). Hyaluronan synthases. *The Journal of Biological Chemistry*, *272*, 13997–14000.

Yamada, Y., Itano, N., Zako, M., Yoshida, M., Lenas, P., Niimi, A., et al. (1998). The gene structure and promoter sequence of mouse hyaluronan synthase 1. *The Biochemical Journal*, *330*, 1223–1227.

Zhu, D., & Bourguignon, L. Y. (1998). The ankyrin-binding domain of CD44s is involved in regulating hyaluronic acid-mediated functions and prostate tumor cell transformation. *Cell Motility and the Cytoskeleton*, *39*, 209–222.