Chapter 2

LncRNAs as Biomarkers for Melanoma

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Additional information is available at the end of the chapter

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Abstract

Melanoma is the most aggressive and serious type of skin cancer. Known for being highly malignant and metastatic, melanoma typically has low survival rates. Prognosis can be improved with an early diagnosis and a good monitoring of the disease. However, current melanoma biomarkers display severe limitations, making them inadequate for early detection of the malignancy. Therefore, it is of urgent matter for us to characterize and establish novel biomarkers with a direct application to daily clinics in order to accurately detect early american joint committee on cancer (AJCC) stages in melanoma patients, efficiently monitor the disease progression, and reliably predict the response to therapies, survival, and likely future recurrence. Long non-coding RNAs (lncRNAs) are a promising biomarker and regulator of tumor progression for many cancers. They are secreted into the bloodstream inside exosomes by a wide range of malignant cells and several of them have actually been validated as promising circulating molecular signatures of other cancer types, but not melanoma. However, in recent years there has been much research into lncRNA melanoma biomarkers, and many of them have been characterized as potentially clinically relevant.

Keywords: melanoma, biomarkers, lncRNAs, cancer, metastatic melanoma

1. Introduction

Melanoma is the most aggressive and serious type of skin cancer. Its propensity for rapid development and ease of metastasis to vital organs such as the brain, lungs, and liver make it so deadly. Additionally, the incidence of melanoma in the United States has been consistently increasing since at least the 1970s [1]. Most importantly, early diagnosis predicts longer survival and better prognosis [2, 3]. This makes efficient and accurate diagnosis of melanoma a priority for clinicians. Thus, the continued exploration for accurate and efficacious biomarkers is a priority among cancer research.

This chapter aims to describe various characterized and novel long non-coding RNAs (lncRNAs) as melanoma biomarkers. We will first explore the shortcomings and problems of current biomarkers and how lncRNAs can serve as the potential future for melanoma markers. We will then look at already characterized lncRNAs such as BRAF-activated non-coding RNA (BANCR), Sprouty 4 (SPRY4), HOX transcript antisense RNA (HOTAIR), Metastasis-associated lung adenocarcinoma transcript (MALAT), and Antisense non-coding RNA in the INK4 locus (ANRIL), as well as current research methods. Finally, we will discuss future perspectives and what we still need to do to adapt lncRNA for use as a melanoma biomarker.

2. The importance of early diagnosis

Melanoma is diagnosed as an AJCC stage I, II, III, or IV. Stages I and II are characterized as melanomas of varying Breslow thicknesses and possible ulceration, but with no lymph node involvement or metastases. Depending on the sub-stage, 5-year survival ranges from 53% to a robust 97% [4]. Stage III is characterized by regional metastases with 5-year survival rates of 40–78% depending on sub-stage [4]. Stage IV is characterized by distant metastases with extremely poor prognosis and 1-year survival rates ranging from 33 to 62% depending on location of metastases and serum LDH level [4].

There have been recent breakthroughs in melanoma treatments for stages IIIC and IV. Common therapies approved by the Food and Drug Administration include both immunotherapy and small molecule targeted therapy. Both immunotherapy drugs such as pembrolizumab (anti-PD-1) [5, 6], nivolumab (anti-PD-1) [7], and ipilimumab (anti-CTLA-4) [7, 8], and small molecules inhibitors such as dabrafenib (BRAF inhibitor) [9], vemurafenib (BRAF inhibitor) [10], and trametinib (MEK inhibitor) [11] have improved patient survival. However, most tumors become drug-resistant shortly after commencing therapy, resulting in disease progression [12, 13]. Unfortunately, our current therapies are more of a temporary stay than a permanent cure.

Thus, the best way to ensure long-term survival is to diagnose the malignancy while it is in its early stages and slow disease progression through surgery and adjuvant therapy. Melanoma biomarkers play an important role in the diagnosis and prediction of the progression of the disease. However, they have severe limitations in regard to precision to detect early stages of melanoma and reliability as a predictor of disease prognosis and treatment response. By understanding the molecular basis of the disease more, we can identify novel biomarkers that can be used to more efficiently diagnose disease which will undoubtedly improve outcomes and quality of life.

3. Current biomarkers in melanoma

According to the National Institutes of Health Biomarkers Definitions Working Group, biomarkers, or "biological markers," are "a characteristic that is objectively measured and

evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [14]. Biomarkers are commonly used in both clinical trials and clinical practice because of its multitude of applications: as diagnostics for identifying patient conditions or diseases, as a tool for staging and characterization of disease, as an indicator of disease progression, or as a predictor of treatment response [14].

Tumor markers are specifically biomarkers of cancer. They are usually proteins that are tumor-derived (produced by the tumor cells) or tumor-associated (produced by the body in response to tumor cells development) [15]. Tumor markers can appear in a variety of samples, but the most commonly used specimens for detection are blood serum and urine. Both are viable for these purposes because of the ease of collection and processing, as well as the secretion of distinctive cancer markers into these fluids.

Proteins are the most common tumor marker because of their central roles in cell signaling and influence on key molecular pathways in various cells and systems of the body. They are often most easily measurable through immunohistochemistry as certain protein levels change between normal and tumor cells. However, many other molecules can also serve as tumor markers. Cell-free circulating tumor DNA (ctDNA) were characterized in the late 1980s and since then, it has been found that ctDNA is correlated with tumor size and disease activity [16, 17]. ctDNA can be analyzed through liquid biopsies and PCR-based assays [18]. Circulating tumor cells (CTCs) are often present in advanced neoplasias that have metastatic potential and can be purified from serum on the basis of different cell surface markers compared to normal blood cells [18, 19]. Serum metabolites are small molecules secreted by tumor cells that can often serve as a signature of the tumor [20–22]. Carbohydrate expression also changes during oncogenic transformation and cancer progression and can serve as a measure of cancer stage [23–25].

This chapter will specifically examine RNA as biomarkers. In recent years, differing transcriptomes among tumor and healthy cells have become a point of emphasis. Several cancers have different noncoding RNA expression profiles in a time- and tissue-dependent manner. Changes in miRNA within a specific tissue has been shown to correlate with disease status including tumor invasiveness and metastatic potential in various cancers such as breast, colorectal, hepatic, lung, pancreatic, and prostate [26]. LncRNAs can also be used as serum tumor markers, which we will examine in detail in the following section.

4. lncRNAs as biomarkers for cancer

Much of our genome codes for RNA with no protein-coding potential. Such RNA is known as noncoding RNA. Long non-coding RNAs (lncRNAs) specifically refer to transcripts longer than 200 bp in length, and can reach up to over tens of kilobases in length. lncRNAs play a vital role in the regulation of many cellular processes especially gene-expression and post-transcriptional activity. This is in part due to their structural versatility and ability to form ribonucleoprotein complexes (RNPs) [27].

It has been noticed that lncRNA expression differs among normal and cancer cells, making it a prime candidate for novel cancer research. Different studies have shown the diverse roles that lncRNAs play in cancer, helping malignant cells proliferate, resist apoptosis, evade growth suppression, maintain genomic instability, and invade and metastasize [28, 29].

What makes lncRNA so valuable as a potential biomarker is its accessibility and detection outside of the cell and in easily collectable biological samples such as blood and urine. LncRNAs are often found in high concentrations in exosomes [30], small cell-derived vesicles 30–100 nm in diameter that are released from the plasma membrane to the extracellular environment. Thus, exosomes contain cytoplasm surrounded by a phospholipid bilayer, along with endosomal compartments known as multivesicular bodies (MVBs) that fuse with the plasma membrane before exosome release [31]. Exosomes contain many particles within the cytoplasm such as proteins and various nucleic acids like mRNA, lncRNA, and miRNA [31]. They are also able to communicate with both the immediate extracellular environment and distant sites for potential metastases in the case of cancer cell exosomes [32]. Additionally, studies have demonstrated that compared to normal cells, cancer cells secrete more and differently constituted exosomes [31, 33, 34].

Exosomes can transfer molecules from cell to cell, and often, they end up in blood or waste to be excreted. It is simple to isolate these vesicles from blood serum or urine, and then analyze its components. Thus, we can use these molecules, including lncRNAs, as a tumor fingerprint to identify potentially tumorigenic cells. The use of exosome particles to identify cancers has already been demonstrated in gastric [35] and pancreatic cancer [36], among others. These circulating lncRNA have already been shown to have great potential as biomarkers for several cancers, which make it all the more promising that some lncRNA can be effective in diagnosing and monitoring melanoma. For example, the expression of the gene $DD3^{PCA3}$ is highly upregulated in prostate cancer cells compared to normal cells [37]. Traditionally, prostate specific antigen (PSA) is a protein biomarker used to test for prostate cancer. However, because the $DD3^{PCA3}$ test has better specificity, it is now used in conjunction with PSA testing (along with TMPRSS2:ERG fusion RNA) to form a more specific test for this cancer. Other examples include MALAT1 in lung cancer, H19 and LINC00152 in gastric cancer, and HOTAIR in colorectal cancer and oral squamous cell cancer [38].

5. Potential lncRNAs as biomarkers for melanoma

Currently, lncRNAs are being used as biomarkers in many different malignancies, as outlined in the previous section. However, there is currently no reported usage of lncRNAs as melanoma biomarkers in a clinical setting. Various lncRNAs are still being tested as potentially viable clinical melanoma biomarkers, including many lncRNA upregulated in and/or used as a tumor marker for other cancers. A brief overview of each is provided here and a summary in **Table 1**. Known mechanisms of action are presented in **Figure 1A–I**.

Melanoma lncRNA	Full name	Function
ANRIL	Antisense non-coding RNA in INK4 locus	Promotes EMT and metastasis
BANCR	BRAF-activated non-coding RNA	Implicated in cell survival, proliferation, and metastasis, expression correlated with disease progression
CASC15	Cancer susceptibility candidate 15	Increases migration and metastatic activity, prognosticator of melanoma stage
HOTAIR	HOX transcript antisense RNA	Promotes EMT and metastasis, prognostic role and serum marker in other cancers
Llme23	–	Inhibits tumor suppressor; plays oncogenic role
MALAT-1	Metastasis-associated lung adenocarcinoma transcript 1	Migration and metastasis, overexpression activates MAPK, Wnt/beta-catenin
SAMMSON	Survival associated mitochondrial melanoma-specific oncogenic non-coding RNA	Promotes cancer cell survival
SLNCR1	–	Promotes EMT and invasion
SNHG5	SnoRNA host gene 5	Melanoma invasion and metastasis
SPRY4-IT1	–	Promotes melanoma cell growth and invasion and blocks apoptosis
UCA-1	Urothelial carcinoma-associated 1	Promotes invasion and metastasis

Table 1. Summary of melanoma lncRNA.

5.1. Antisense non-coding RNA in the INK4 locus (ANRIL)

Antisense non-coding RNA in the INK4 locus (ANRIL) is a 3834 nt lncRNA consisting of 19 exons alternatively spliced in the antisense direction of the p15/CDKN2B-p16/CDKN2A-p14/ARF gene cluster on chromosome 9 in humans. ANRIL interacts with polychrome repressive complexes 1 and 2 (PRC1/2) to reduce the expression of essential tumor suppressor proteins p15INK4b and p16INK4a. It has been shown that ANRIL can promote epithelial to mesenchymal transition (EMT) and metastasis in cancers such as non-small cell lung carcinoma and pancreatic [39, 40]. Although a research group used genome wide association studies (GWAS) to determine several single nucleotide polymorphisms in the ADP ribosylation factor (ARF) locus, including one within the ANRIL sequence, rs1011970, is associated with melanoma, we know little else about ANRIL's potential role in melanoma. As such, further investigation is needed to determine its suitability as a melanoma biomarker.

5.2. BRAF-activated non-coding RNA (BANCR)

BRAF-activated non-coding RNA (BANCR) is a 693 bp lncRNA transcript highly induced by oncogenic BRAF and overexpressed in melanoma. More than 70% of melanomas contain an

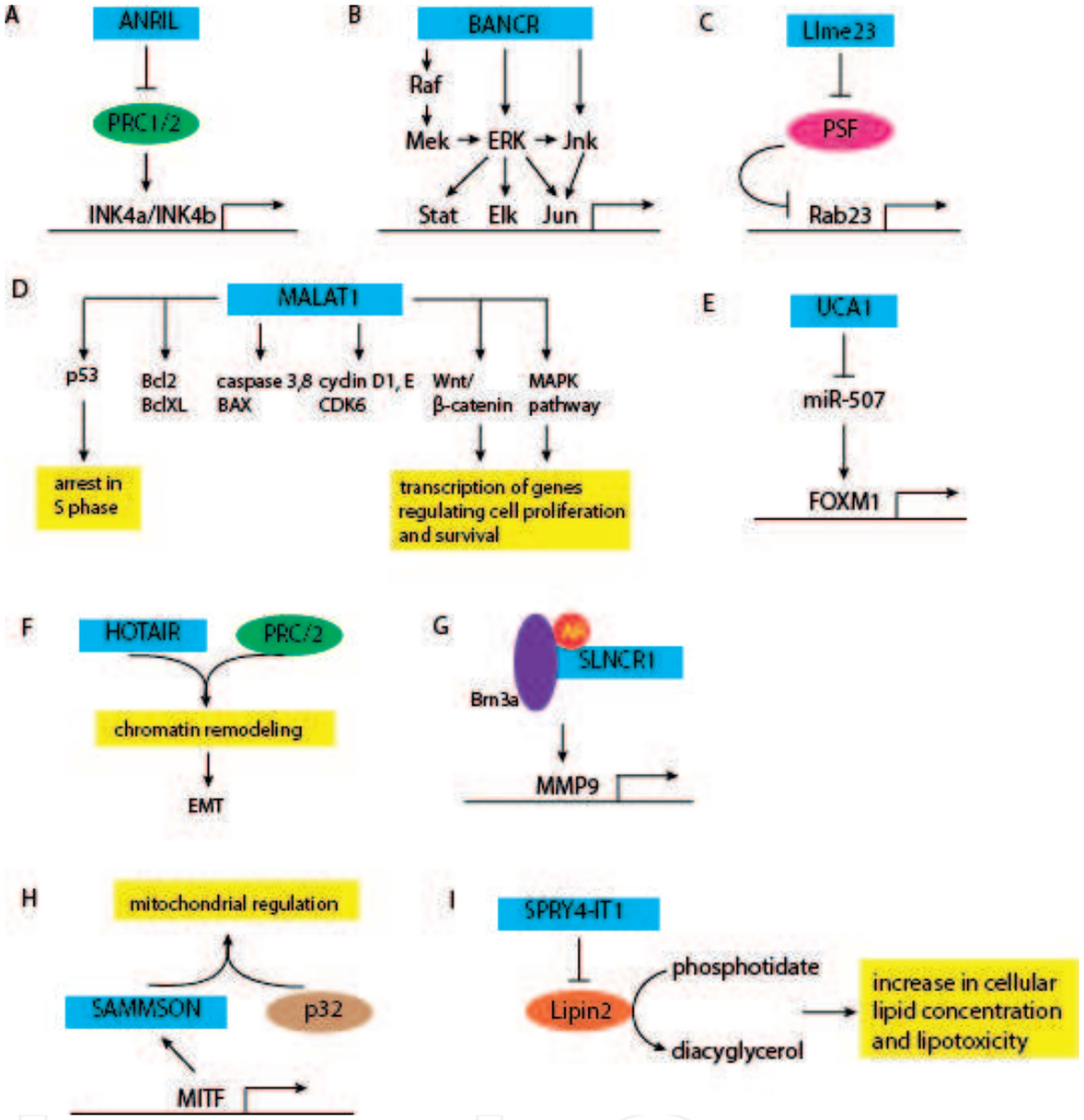

Figure 1. This figure displays the pathways and mechanisms of action of potential melanoma lncRNAs. Growth and proliferation: ANRIL, BANCR, Llme23, MALAT-1, UCA1 (A–E). Invasion and metastases: HOTAIR, SLNCR1 (F–G). Apoptosis: SAMMSON, SPRY4-IT1 (H–I). Mechanism not yet characterized: SNGH5, CASC15.

activating BRAF mutation, of which 90% is the V600E mutant. Mutant BRAF causes the upregulation of various proteins and transcripts implicated in cell survival, proliferation, and metastasis, including BANCR. BANCR is overexpressed in both melanoma cell lines and tissues, with increased expression correlated with disease progression [41, 42]. Flockhart et al. first characterized BANCR as regulating melanoma cell migration [41]. Perhaps unsurprisingly, patients with high levels of BANCR had a much lower survival rate than patients with low levels of BANCR (3-year survival of 40% compared to 71%) [42]. So far, research on BANCR has shown its potential value as both a target for therapy as well as a prognostic measure.

However, there have been few studies to validate its efficacy. Additionally, its presence in serum, which would make it a more viable biomarker, has not yet been demonstrated.

5.3. Cancer susceptibility candidate 15 (CASC15)

Cancer susceptibility candidate 15 (CASC15) is a 530 kb intergenic lncRNA (lincRNA) on chromosome six that was observed to be actively transcribed in metastatic melanoma. Its expression increases migration and metastatic activity, although its mechanism of action and binding partners is currently unknown [43]. A study found that CASC15 was both expressed in melanoma cell lines and upregulated in a mouse xenograft model of brain metastases [44]. Additionally, CASC15 can serve as a good prognosticator of melanoma stage, as levels increase during melanoma progression, with significant expression in advanced stage IV metastases compared to controls and melanoma in situ (MIS) [44]. However, while normal tissue shows virtually undetectable levels of CASC15, other cancers can also show high levels of CASC15 expression, making it less specific than other biomarkers [44]. Nonetheless, if it is indeed possible to isolate CASC15 in blood or urine samples of melanoma patients, which to date has not been done, it can serve as a valuable diagnostic and prognosticator of metastatic melanoma.

5.4. HOX transcript antisense RNA (HOTAIR)

HOX transcript antisense RNA (HOTAIR) is a 2.2 kilobase lncRNA in an intronic region of the HOXC gene locus. HOTAIR is believed to regulate gene expression through chromatin remodeling; it promotes epithelial-mesenchymal transition (EMT) and cancer metastasis by coordinating with polycomb repressive complex 2 (PRC2) to repress the expression of various genes suppressing metastasis [45, 46]. Its net effect in metastatic melanoma is to promote motility, invasion, and metastatic potential [47], evidenced by its upregulation in metastatic tissue. HOTAIR's role as a prognostic factor in breast cancer is well characterized, and it may also serve prognostic roles in gastroenteric tumors and liver metastases [45]. Additionally, its potential as a serum biomarker in other cancers has been documented [48, 49]. It may serve a similar capacity in melanoma, but more research still needs to be done, including verification of its presence in serum.

5.5. Llme23

Llme23 is a 1600 bp lncRNA expressed exclusively in melanoma. It was first discovered through identifying lncRNA binding partners to polypyrimidine tract-binding (PTB) protein-associated splicing factor (PSF) via RNA-SELAX affinity chromatography [50]. PSF is a tumor suppressor repressing the transcriptional activity of multiple proto-oncogenes, including Rab23 [50]. Llme23 interacts with and subsequently blocks the function of PSF, thus inhibiting its tumor suppressor function [50]. Because of its exclusive nature to melanoma cell lines, Llme23 would serve as a highly specific biomarker for melanoma. Before it can be adapted to clinical use, further research is required to determine its secretion and levels in plasma and/or urine.

5.6. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1)

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1), also known as nuclear-enriched transcript 2 (NEAT2) is a well-characterized, roughly 8000 bp lncRNA. It has been extensively studied and shown to have a regulatory function lung cancer cell migration and metastasis [51], as well as similar roles in liver cancer [52], thyroid cancer [53], and neuroblastoma [54]. MALAT-1 targets many tumor suppressor genes, and its overexpression activates MAPK, Wnt/beta-catenin, p53, caspase-3, caspase-8, and the Bax signaling pathway. Its relevance to melanoma has recently come to attention, as Tian et al. found that MALAT-1 is highly expressed in melanoma tumors compared to normal tissues [55]. Moreover, metastatic sites display higher levels of MALAT-1 than primary tumors [55]. MALAT-1 is a promising new biomarker for melanoma, as its presence in serum and urine has already been reported in prostate cancer patients [56, 57].

5.7. Survival associated mitochondrial melanoma-specific oncogenic non-coding RNA (SAMMSON)

Survival associated mitochondrial melanoma-specific oncogenic non-coding RNA (SAMMSON) is located around 30 kb downstream of microphthalmia-associated transcription factor (MITF), an oncogene specific to melanoma. Although SAMMSON and MITF are only co-amplified in around 10% of melanomas, SAMMSON itself was detected in 90% of melanoma samples from the TCGA RNA-seq data set [58]. SAMMSON binds to p32, which regulates tumor metabolism through balancing oxidative phosphorylation and glycolysis [58, 59]. Knockdown of SAMMSON resulted in decreased cell viability and apoptosis. SAMMSON localizes to the mitochondria, so its presence in serum may be limited. However, further investigations should be done to confirm its potential as a biomarker.

5.8. SLNCR1

SLNCR1 is a lncRNA that promotes melanoma invasion and is associated with survival outcome. The brain-specific homeobox protein 3a (Brn3a) and androgen receptor (AR) binds to SLNCR1 to increase melanoma invasion by activating the transcription of MMP9, required for the degradation of extracellular matrix during EMT and invasion [60]. Interestingly, the higher incidence of melanoma metastases in males compared to females may be explained by ARs being binding partners for SLNCR1 [60]. Again, there is presently no research on the presence of SLNCR1 in plasma and thus its viability as a melanoma biomarker.

5.9. SnoRNA host gene 5 (SNHG5)

SnoRNA host gene 5 (SNHG5) is a 524 bp lncRNA whose levels were found to be significantly higher in the serum of patients with melanoma compared to normal subjects as well as patients with squamous cell carcinoma [61]. This suggests that this lncRNA plays a role in

melanoma formation and/or metastasis. Its presence in serum is already documented, making it a promising biomarker. However, much more research needs to be done as its mechanism in melanoma has yet to be characterized and the only study looked at just 24 patients with malignant melanoma [61].

5.10. SPRY4-IT1

SPRY4-IT1 is a lncRNA located within the intron of the Sprouty 4 (SPRY4) gene. It was first identified as upregulated in melanoma cells compared to melanocyte and keratinocyte controls [62]. SPRY4-IT1 promotes melanoma cell growth and invasion and also blocks apoptosis [62]. It acts by binding to and inactivating lipin 2, an enzyme involved in fatty acid metabolism [63]. Knockdown of SPRY4-IT1 induces an increase of lipids in the cell and can lead to apoptosis due to lipotoxicity [63]. Based on plasma samples of healthy individuals (N = 79) and malignant melanoma patients (N = 70), SPRY4-IT1 expression was significantly higher in malignant melanoma patients. Additionally, elevated SPRY4-IT1 significantly reduced patient survival rate and is strongly associated with more advanced tumor stage [64]. These data suggest that SPRY4-IT1 may be used as a marker for both diagnosis and staging. SPRY4-IT1 was also found to be present in the plasma of esophageal squamous cell carcinoma patients, and associated with poor prognosis [65]. Limitations of its efficacy as a melanoma biomarker include specificity, as it is also found to play roles in prostate cancer [66], glioma [67], and gastric cancer [68], among others.

5.11. Urothelial carcinoma-associated 1 (UCA-1)

Urothelial carcinoma-associated 1 (UCA-1) is a 1.4 kb lncRNA implicated in several cancers including breast, gastric, and pancreatic [69–71]. A recent study by Wei et. al. showed that UCA-1 is upregulated in melanoma as well [72]. It acts by inactivating miR-507, which in turn leads to the upregulation of transcription factor FOXM1 mRNA and invasion and metastasis [72]. Studies have shown that urine UCA-1 is a possible diagnostic biomarker in bladder cancer [73], though it has not yet been tested as a marker for melanoma.

6. Research and clinical methods

One common method of identifying novel lncRNAs is by RNA-seq expression profiles between cancer cells and normal cells expressing relevant oncogenes [41]. BANCR was discovered through this method. Previously, Sanger sequencing of cDNA libraries and tiling arrays were the preferred method for identifying lncRNA, but they have since been replaced by RNA-seq and other next generation sequencing technologies [74].

To characterize the mechanism of lncRNA action, it is important to determine subcellular localization and binding partners. To determine the subcellular localization of a particular, fluorescence in situ hybridization (FISH) may be used. To find molecular interactions

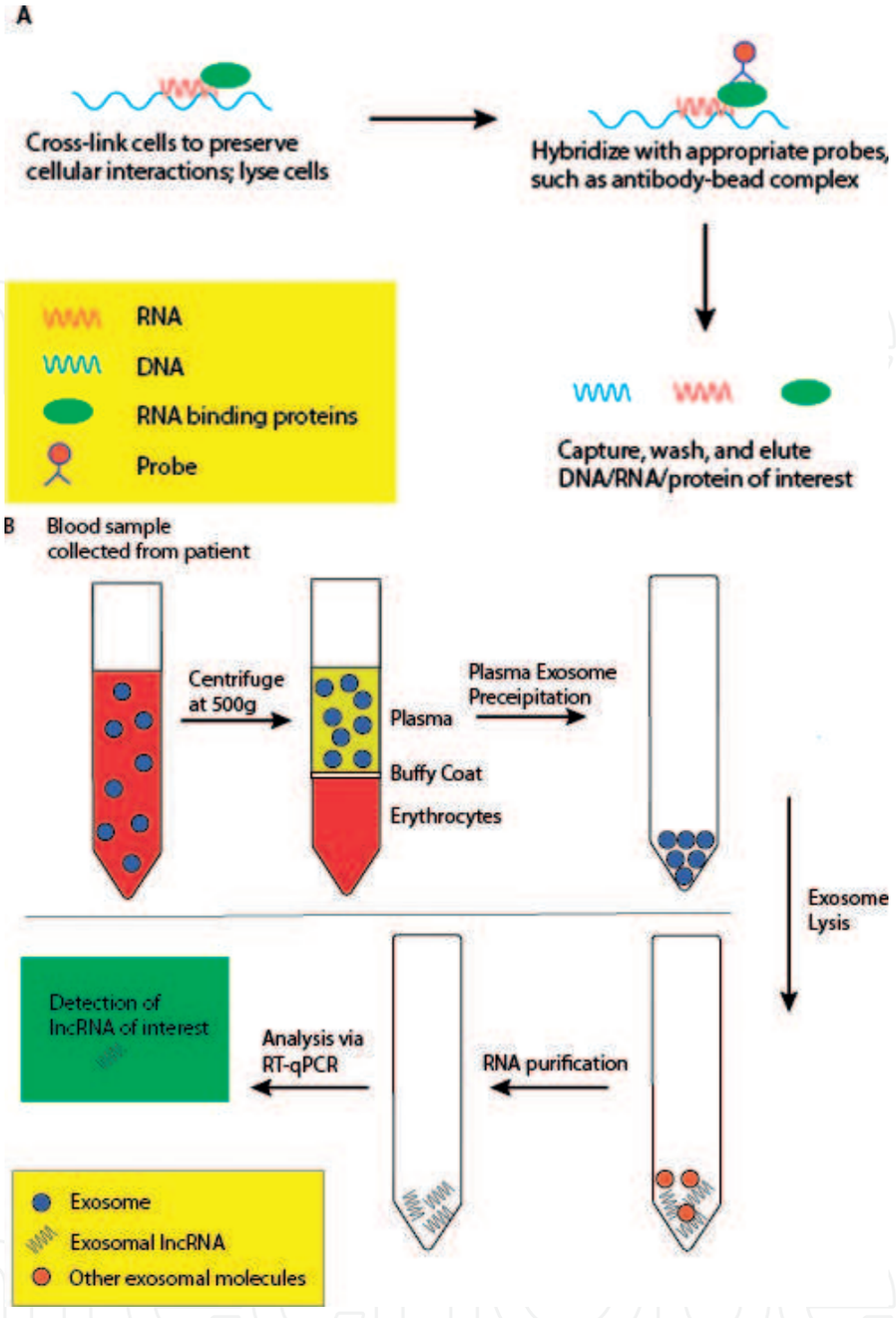

Figure 2. Experimental techniques. (A) General workflow for immunoprecipitations. Many of these pull-down experiments to find RNA binding partners follow a similar protocol, including ChIRP, RAP RNA, and RIP. RNA-DNA: ChIRP, RAP, CHART. RNA-RNA: RAP RNA, CLASH. RNA-protein: RIP, CLIP. (B) Clinical detection of circulating lncRNA. lncRNA are found in serum exosomes. First, a blood sample collected from a patient is centrifuged to separate the plasma. Then, exosomes are precipitated from the plasma, lysed, and RNA collected and purified. This purified RNA can then be analyzed via RT-qPCR to identify any lncRNAs of interest.

between the lncRNA and DNA, RNA, and proteins, a variety of pull-down experiments are performed (**Figure 2A**). To determine RNA-DNA interactions, chromatin isolation by RNA purification (ChiRP) is most commonly used. Other methods include RNA antisense purification (RAP), which uses RNA antisense probes to map RNA interactions with chromatin

[75], and capture hybridization analysis of RNA targets (CHART), which uses capture oligonucleotides that hybridize to the RNA of interest, which is then enriched alongside its targets [76]. To determine RNA-RNA interactions, RAP RNA or cross-linking, ligation, and sequencing of hybrids (CLASH), may be used. RAP RNA uses differential cross-linking with psoralen for RNA-RNA interactions and formaldehyde for protein-mediated RNA-RNA interactions [77]. CLASH, a less labor-intensive method, uses UV cross-linking and sequencing of ligated RNA-RNA hybrids, whereupon chimeric reads are identified using bioinformatics [78]. To determine RNA-protein interactions, RNA immunoprecipitation (RIP) can be used to analyze RNA associations with proteins at specific time points [79], while cross-linking immunoprecipitation (CLIP) maps RNA-protein interactions through in vivo UV cross-linking of cells [80].

Clinically, the detection of circulation lncRNA for use in diagnostics and monitoring would be simple (**Figure 2B**). First, plasma exosomes would need be isolated from a patient's blood sample. Centrifugation of the blood sample first separates the plasma from the cellular components, and the exosomes can then be precipitated from the plasma using one of several techniques: ultracentrifugation, size-exclusion chromatography/ultrafiltration, immunoaffinity capture-based technique, or use of a commercial, exosome precipitation kit [81]. Ultracentrifugation involves spinning the sample at forces up to 1,000,000 g, and is considered the most effective and most used method [81]. Second, RNA extraction for lncRNA analysis would need to be done. The exosomes are lysed, RNA extracted and purified, and then analyzed using qRT-PCR.

7. Conclusion: the future of lncRNAs in melanoma as biomarkers and targets for therapy

Overall, lncRNAs serve as promising biomarkers for melanoma, though much more research needs to be done on them before they can be used clinically. The presence of lncRNA in blood and urine make them particularly valuable to the field of cancer diagnostics as presently, there is a dearth of early diagnostic measures for melanoma. Currently, potential melanomas must be detected by a patient or physician. The major shortcoming of this is that sometimes malignant melanomas do not appear obvious until it is at a late stage, and patients themselves often cannot identify harmful lesions at early stages. Additionally, it is difficult to keep track of potentially malignant nevus in certain areas of the body. Once a potentially tumorigenic nevus or lesion is clinically observed, the first line of diagnostics is the histopathology of biopsies, which are both invasive and expensive.

Diagnoses using circulating lncRNA could serve as an improvement to these biopsies. Not only are they minimally invasive, they are also less expensive and can be conducted at regular intervals for high-risk patients (those with a melanoma in the past 5 years, certain genes, phenotypic red hair, Irish-Scottish ancestry, high mole count, frequent sun exposure, etc.). Moreover, many lncRNAs can also provide valuable prognostic information, including progression, staging, and size to tumor.

Some concerns for the use of lncRNA as biomarkers do exist. The lncRNA must be present in sufficient quantity for it to be able to be detected and analyzed using standard methods. Additionally, as discussed previously, many of these lncRNAs are also upregulated in other cancers, lowering its specificity as a melanoma biomarker. However, this may not be a bad thing, as other malignancies may be able to be "accidentally" detected.

Certain lncRNA can also be used as targets for novel therapies. LncRNAs like BANCR and MALAT-1 are responsible for cell migration and metastases. Targeting or knocking down these lncRNA in vivo may prevent further progression and invasion of early stage melanomas and limit the metastatic activity of late stage melanomas.

In conclusion, lncRNAs are likely to be suitable melanoma biomarkers for a variety of reasons: (1) They are secreted into the bloodstream and easily accessible for analysis using non-invasive and inexpensive methods. Because they are secreted within exosomes, they are also protected from various RNases within the bloodstream. (2) Various lncRNAs are secreted at different time-points of disease progression. Those secreted early on have valuable diagnostic potential while others may be useful in determining disease development and prognosis. (3) LncRNAs are generally highly specific for melanoma, a shortcoming of current protein biomarkers. (4) Noncoding RNAs are responsible for a variety of cellular functions and implicated in many important pathways, making them valuable prognosticators of disease. (5) LncRNA biology is still a relatively novel field, which holds a lot potential as more research is being conducted.

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