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Meeting the needs of breast cancer: A nucleolin's perspective

Ana C. Gregório[a,b], Manuela Lacerda[c], Paulo Figueiredo[d], Sérgio Simões[a,e], Sérgio Dias[f], João Nuno Moreira[a,e,*]

[a] CNC – Center for Neurosciences and Cell Biology, University of Coimbra, 3004-504 Coimbra, Portugal
[b] IIIUC – Institute for Interdisciplinary Research, University of Coimbra, 3030-789 Coimbra, Portugal
[c] IPATIMUP – Institute of Molecular Pathology and Immunology, University of Porto, 4200-465 Porto, Portugal
[d] IPOFG-EPE – Portuguese Institute of Oncology Francisco Gentil, 3000-075 Coimbra, Portugal
[e] FFUC – Faculty of Pharmacy, Pólo das Ciências da Saúde, University of Coimbra, 3000-354 Coimbra, Portugal
[f] IMM – Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, 1649-028 Lisbon, Portugal

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ABSTRACT

A major challenge in the management of breast cancer disease has been the development of metastases. Finding new molecular targets and the design of targeted therapeutic approaches to improve the overall survival and quality of life of these patients is, therefore, of great importance. Nucleolin, which is overexpressed in cancer cells and tumor-associated blood vessels, have been implicated in various processes supporting tumorigenesis and angiogenesis. Additionally, its overexpression has been demonstrated in a variety of human neoplasias as an unfavorable prognostic factor, associated with a high risk of relapse and low overall survival. Hence, nucleolin has emerged as a relevant target for therapeutic intervention in cancer malignancy, including breast cancer. This review focus on the contribution of nucleolin for cancer disease and on the development of therapeutic strategies targeting this protein. In this respect, it also provides a critical analysis about the potential and pitfalls of nanomedicine for cancer therapy.

1. Introduction

Nucleolin has emerged in the cancer scene as a potential target. Nucleolin participates in various cellular functions controlling different components of RNA and DNA metabolism, including ribosome biogenesis, ribosomal (r)RNA maturation, ribosomal (r)DNA transcription and chromatin structure (Ginisty et al., 1999; Srivastava and Pollard, 1999). Therefore, nucleolin is mainly located at the dense fibrillar and granular regions of the nucleolus and nucleoplasm (Lischwe et al., 1981). Nonetheless, different pools of nucleolin, in the cytoplasm and cell membrane, have also been demonstrated to exhibit important functions (Srivastava and Pollard, 1999; Borer et al., 1989; Semenkovich et al., 1990). Altogether, nucleolin's biological functions that contribute to cell homeostasis are also responsible for its role in the development of malignant traits under pathological conditions. In this respect, overexpression of nucleolin, and increased localization at the cell membrane, have been identified in different cancer cell lines (Semenkovich et al., 1990; Turck et al., 2006; Fonseca et al., 2015; Moura et al., 2012; Krust et al., 2011a; Benedetti et al., 2015; Destouches et al., 2011) and endothelial cells (Christian et al., 2003; Porkka et al., 2002; Birmpas et al., 2012a,b), as well as in tumors of diverse histological origin (Galzio et al., 2012; Hoja-Lukowicz et al., 2009; Qiu et al., 2013; Wu et al., 2014; Grinstein et al., 2002; Guo et al., 2014; Xu et al., 2016; Peng et al., 2010; Ridley et al., 2008). Nucleolin overexpression contributes to tumorigenesis and cancer progression by supporting cancer cells proliferation and survival, and by promoting invasion and angiogenesis. Altogether, this has generated an increased interest in nucleolin as a therapeutic target, and prompted the development of various strategies targeting this protein.

This manuscript aims at reviewing the pathological roles of nucleolin, and its clinical implications in cancer disease, particularly in the setting of breast cancer. Finally, the strategies targeting nucleolin for cancer treatment, within the frame of the pitfalls of nanomedicine-based therapy, will also be addressed.

Abbreviations: AML, acute myeloid leukemia; DFS, disease-free survival; DLBCL, diffuse large B-cell lymphoma; DSPC, 1,2-distearoyl-sn-glycero-3-phosphocholine; DSPE, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine; EPR, enhanced permeation and retention; MB, molecular beacon; MTP-PE, Muramyl tripeptide phosphatidyl ethalonamine; PDAC, pancreatic ductal adenocarcinoma; PEG, poly(ethylene glycol); R-CHOP, rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine, prednisolone combination chemotherapy; TCGA, The Cancer Genome Atlas

* Corresponding author at: Center for Neuroscience and Cell Biology, University of Coimbra, Faculty of Medicine (Pólo I), Rua Larga, 3004-504 Coimbra, Portugal.
E-mail address: jmoreira@ff.uc.pt (J.N. Moreira).

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Table 1
Cellular targets of nucleolin and implications in cancer disease.

Molecular mechanism	Target	Implication in cancer disease	References
mRNA stabilization	*HIF-1α*	Proliferation, invasion, angiogenesis	(Cheng et al., 2014; Semenza, 2010)
	MMP9	Migration, invasion, angiogenesis	(Hsu et al., 2015; Gialeli et al., 2011)
	MMP2	Migration, invasion	(Gialeli et al., 2011; Jin et al., 2015)
	Egfr	Proliferation, survival, differentiation	(Xie et al., 2016; Normanno et al., 2006)
	Il10	Anti-apoptotic, immunosurveillance	(Hsu et al., 2016; Hamidullah and Konwar, 2012)
	BCL2	Survival (anti-apoptotic)	(Ishimaru et al., 2009; Ishimaru et al., 2010; Willimott and Wagner, 2010; Sengupta et al., 2004; Zhang et al., 2010)
	IL2	Immunosurveillance	(Chen et al., 2000; Boyman and Sprent, 2012)
	GAST	Proliferation, invasion, survival (anti-apoptotic)	(Lee et al., 2007; Ferrand and Wang, 2006)
mRNA translation suppression	*TP53*	Tumor suppressor	(Takagi et al., 2005; Muller and Vousden, 2014)
mRNA translation enhancement	*MMP9*	Migration, invasion, angiogenesis	(Gialeli et al., 2011; Fahling et al., 2005)
	AKT1	Proliferation, survival (anti-apoptotic)	(Abdelmohsen et al., 2011; Hers et al., 2011)
	VEGF-D	Lymphatic dilatation, metastases	(Morfoisse et al., 2016)
	CCN1	Survival (anti-apoptotic)	(Abdelmohsen et al., 2011)
Transcription enhancement	*Il9r*	Immunosurveillance	(Shang et al., 2012)
	VEGF	Angiogenesis	(Uribe et al., 2011)
	IRF2	Proliferation	(Masumi et al., 2006)
	CD59	Modulation of immune responses (inhibitor of reactive lysis)	(Tediose et al., 2010)
	BCL221	Survival (anti-apoptotic)	(Tediose et al., 2010)
	MCL1	Survival (anti-apoptotic)	(Tediose et al., 2010)
	E6 and *E7*	Target p53 for degradation, maintenance of viral (HPV) episomes	(Grinstein et al., 2002; Oh et al., 2004)
Biogenesis	*miR-15a/16*	Pro-apoptotic	(Pickering et al., 2011)
	miR-21, miR-103, miR-221, and *miR-222*	Proliferation, survival (anti-apoptotic), migration, invasion	(Pichiorri et al., 2013)

1.1. Role of nucleolin in tumorigenesis and metastization

In the nucleolus, nucleolin controls chromatin accessibility and dynamics, thus contributing to the regulation of RNA polymerase I transcription activity (Angelov et al., 2006). Moreover, its association with unmethylated rRNA genes interferes with the binding of transcription termination factor 1 to promoter-proximal terminator T0, further contributing for the maintenance of an euchromatin state and the subsequent transcription by RNA polymerase I (Rickards et al., 2007; Cong et al., 2012). Additionally, nucleolin is also a key factor participating in several steps of the assembly and maturation of pre-rRNA (Ginisty et al., 2000; Bouvet et al., 1998; Roger et al., 2003). As a result, nucleolin overexpression enables the sustained proliferation of cancer cells by potentiating a high level of protein synthesis.

Nucleolin also promotes oncogenesis, acting as a transcriptional factor through direct binding to promoter region of target genes (in the nucleoplasm), or by regulating mRNA stability or translation (in the cytoplasm). A complete list of oncogenes and mRNAs under the regulation of nucleolin is summarized in Table 1. Among them, nucleolin and the human heterogeneous nuclear ribonucleoprotein K (hnRNP K) bind selectively to the guanosine (G)- and cytosine (C)-rich sequences of *VEGF* promoter region, respectively, acting as transcriptional activators (Uribe et al., 2011). Nucleolin also participates in human papillomavirus 18 (HPV18)-associated cervical carcinogenesis through the control of HPV18 enhancer chromatin structure. The downregulation of nucleolin protein expression resulted in the inhibition of *E6* and *E7* oncogene transcription and selectively decreased $HPV18^+$ cervical cancer cell growth (Grinstein et al., 2002).

At the mRNA level, nucleolin regulates the expression of several key proteins involved in cancer growth and progression, as BCL2 (Ishimaru et al., 2009; Ishimaru et al., 2010; Sengupta et al., 2004), p53 (Tate et al., 2006), Akt1 (Huang et al., 2006), MMP-9 (Fahling et al., 2005) or MMP-7 (Hsu et al., 2015). BCL2 overexpression in various cancers results from nucleolin binding to the adenine-uracil (AU)-rich element of *BCL2* mRNA, increasing its stability (Ishimaru et al., 2009; Ishimaru et al., 2010; Sengupta et al., 2004). A similar mechanism applies to MMP-9, whose expression increased upon nucleolin binding to the mRNA three prime untranslated region (3′UTR), improving the proteinase translational efficiency (Fahling et al., 2005). More recently, the modulation of mRNA stability by a truncated form of nucleolin, generated through the action of metalloproteinase 7 (MMP-7), was further implicated in the increased expression of several oncogenes, including *MMP9*, and hypoxia inducible factor 1 α (*HIF1α*), while the expression of various tumor suppressors was decreased (Hsu et al., 2015).

Interestingly, VEGF was shown to induce the translocation of nucleolin from the nucleus to the cell membrane, through the activation of a signaling pathway involving receptor protein tyrosine phosphatase β/ζ (RPTPβ/ζ), culminating in nucleolin phosphorylation by PI3K (Koutsioumpa et al., 2015). In fact, phosphorylation regulates nucleolin's subcellular localization, and has been correlated with metastatic potential in various carcinomas (Wu et al., 2014). Pleiotrophin also induced the translocation of nucleolin in a similar manner to that of VEGF (Koutsioumpa et al., 2013), in addition to its direct binding to cell surface nucleolin (Koutsioumpa et al., 2012; Said et al., 2005). At the cell membrane, nucleolin acts as a receptor to different ligands involved in cancer progression, and mediates their mitogenic, anti-apoptotic, invasive and pro-angiogenic effects (Table 2). Cell surface nucleolin also acts as a binding partner for several membrane proteins, such as those of the ErbB tyrosine kinase receptor family (Table 2).

1.2. Nucleolin as a prognostic factor in cancer disease

Over the last decade, the value of nucleolin as a potential prognostic factor has raised much interest. Its overexpression in human-derived tumor tissues has been associated with a higher risk of recurrence and worse overall survival, in a variety of neoplasias (Qiu et al., 2013; Guo et al., 2014; Xu et al., 2016; Ridley et al., 2008; Chen et al., 2015; Zhao et al., 2013; Gilles et al., 2016; Jain et al., 2017; Marcel et al., 2017). Nevertheless, the power of nucleolin overexpression as a biomarker for efficacy apparently depends on the clinical setting. In a multifactorial analysis of outcome predictors in pediatric intracranial ependymoma, the five-year event-free survival was observed in only 31 ± 7% of patients with strong nucleolin immunostained tumors (Ridley et al., 2008). A subsequent study further demonstrated that elevated nucleolin expression (≥ 50% cells of complete section) was associated with