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Original Article

Molecular interaction between small nuclear ribonucleoprotein polypeptide G and heat shock protein 70.14: a microscale thermophoresis exposition towards developing anti-cancer drugs

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Abstract: Background: Targeting protein-protein interactions (PPIs) linked to protein quality control (PQC) pathways as potential anti-cancer drug targets have unanimously widened biological insights and the therapeutic potential of PPIs as smart-drug discovery tools in cancer. PPIs between disease-relevant proteins associated with protein homeostasis in PQC pathways have been linked to improved mechanistic understanding associated with conformational abnormalities and impairment, cellular proteotoxicity, induced apoptosis, and pathogenesis in different types of cancers. In this context, PPIs between small nuclear ribonucleoprotein polypeptide G (SNRPG) and heat shock protein 70.14 (Hsp70.14) have attracted attention as potential smart drug discovery tools in cancer diagnostics and therapeutics. Validated evidence of high-quality biological data has shown the presence of the two proteins in different types of cancers including breast cancer. The links between SNRPG and Hsp70.14 in cancer-cell networks remain elusive, overlooked, and uncharacterized. Methodology: In this study, we explored the interaction between the two oncogenic proteins using the MST-based assays. Results: The results revealed a low K_D in the nanomolar concentration range of 2.4673×10^{-7} demonstrating a great affinity for SNRPG binding to Hsp70.14. Conclusions: The results suggest a possible involvement between the two proteins in hostile tumour microenvironments. Furthermore, these findings offer a different therapeutic perspective that could pave the way for the creation of novel small molecule inhibitors as drugs for the treatment of cancer.

Keywords: Anti-cancer drug discovery, dissociation constant, Hsp70.14, microscale thermophoresis, protein-protein interactions, proteostasis, SNRPG

Introduction

The importunity and peremptory need to alleviate existing cancer challenges have prompted scientists to consider targeting protein-protein interactions (PPIs) associated with protein homeostasis (proteostasis) in protein quality control (PQC) pathways as potential anticancer drug targets [1-3]. The interference of disease-relevant proteins associated with proteostasis in PQC pathways has been linked to mechanistic shrewdness associated with cellular proteotoxicity, induced apoptosis, and subsequent pathogenesis in cancer. The intrinsic proteostatic instability coupled with a hostile tumour microenvironment presents a demanding task for the PQC in oncogenic cells [1-4]. As a result, PQC machineries can effectively regulate general protein turnover as well as the degradation of tumour-promoting or suppressive proteins, which is an indispensable process in modulating tumour development and tumorigenesis [5].

Dysregulation of Smith (Sm) proteins (B/B', D1, D2, D3, E, F, and G) proteostasis has been linked to pathophysiological cues and disease progression in Huntington's, Parkinson's, Alzheimer's, type II diabetes, and different type of cancers [6, 7]. Sm proteins play a pivotal role in the assembly of uridyl-rich small nuclear ribo-

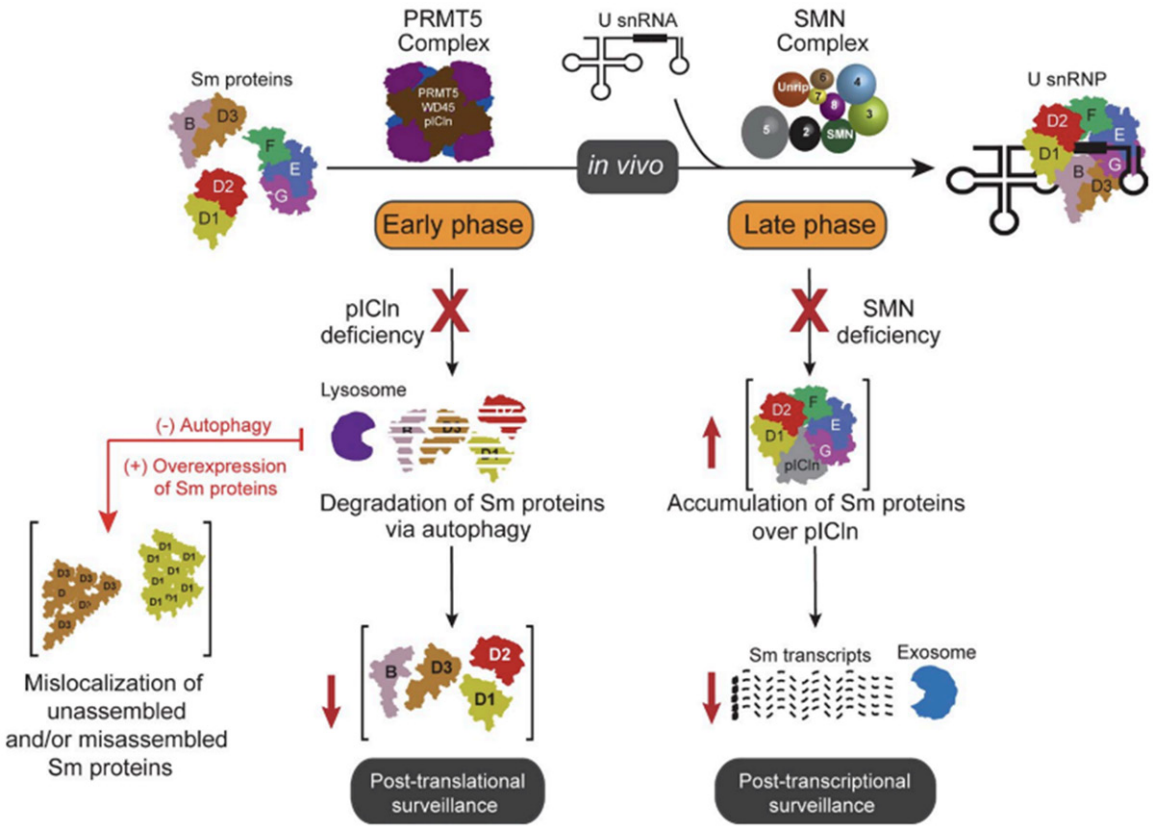

Figure 1. Schematic representation of Sm proteins' regulation in protein quality control (PQC) during U snRNP assembly. Parkinson's disease, Alzheimer's disease, Huntington's disease, as well as type 2 diabetes and cancer and are just a few diseases examples of the efficient homeostasis that prevent cellular proteotoxicity, apoptosis and disease progression. Figure taken from Prusty et al [5].

nucleoprotein particles (U snRNPs; U1, U2, U4, and U5), which are the core components of both the minor and major spliceosomes [5, 6]. Non-small cell lung cancer (NSCLC) cells undergo apoptosis when Sm protein regulation is disrupted [5-8]. Sm proteins are frequently upregulated in NSCLC and their increased expression is positively correlated with disease severity [5-8].

Regardless of the tremendous efforts to uncover multiple aspects of how Sm proteins are regulated by molecular chaperones in PQC systems, the task remains unclear, elusive, and beyond the targeting capabilities of orthodox technologies [5]. Sm proteins lack common sequence motifs that identify with the known chaperones involved in lysosomal proteolysis [9]. The dysregulation of Sm proteins in PQC pathways leads to their improper folding, aggregation, and subsequent degradation via autophagy (shown in **Figure 1**) [5]. Thus, the PQC system is critical for cell survival during unfavorable or harsh conditions like hostile tumour microenvironments. To safeguard themselves against any type of environmental stress, cells produce regulatory housekeeping chaperones in PQC machineries called heat shock proteins (HSPs) [10].

The proteostasis of the core splicing Sm protein, small nuclear ribonucleoprotein polypeptide G (SNRPG), in PQC systems is of particular interest. SNRPG has attracted significant attention in PPI-focused drug technology because of its possible implications for carcinogenesis and tumor progression [8]. Different expression levels of SNRPG have been discovered in a variety of malignancies, suggesting that the core splicing protein amasses in hostile tumour environments, playing an important part in the genesis and development of cancer [8, 11]. PPIs mediate SNRPG, thereby making it a prospective anti-cancer therapeutic target in PPI-focused drug development [8].

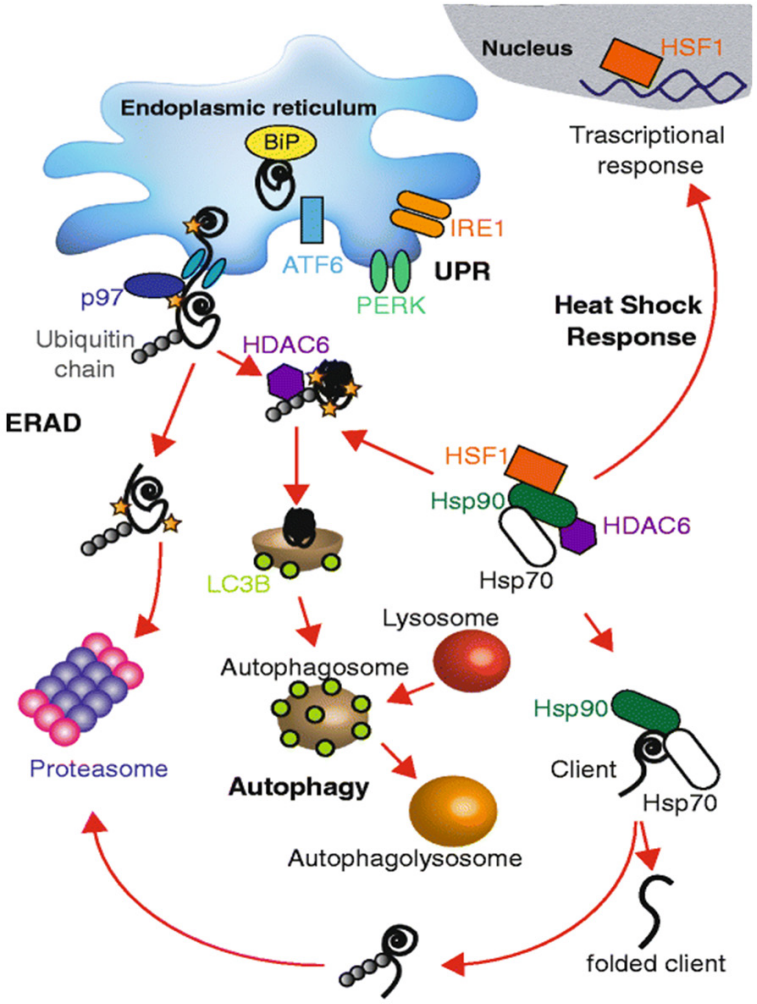

Figure 2. A diagram depicting the regulatory functions of house-keeping chaperones in PQC machineries. Hsp70 is actively involved in protein folding thereby dictating protein homeostasis in cell environments. Dysregulation of proteins and their failure to attain final conformation leads to ubiquitylation and degradation by the proteasome. Figure taken from Sannino and Brodsky [4].

According to Sannino and Brodsky as well as Powers and co-workers [4, 10], hostile tumour environments overexpress regulatory house-keeping chaperones in PQC machineries via autophagy or ubiquitin-proteasome pathway. The most abundant housekeeping chaperone that is overexpressed in different cancer types is a heat shock protein 70 (Hsp70) [4, 12-14]. Hsp70 plays a pivotal role in multi-protein assemblies linked to proteostasis (protein folding, degradation, and activation) in PQC machineries via the autophagy pathway (shown in **Figure 2**) [4, 15]. It is involved in signal transduction pathways or PQC systems with the ability to proofread, identify and repair misfolded and/or improperly-folded proteins [15].

The increase in proliferation of malignant cells has been observed to correlate with the overexpression of Hsp70, whereas its knockdown decreases the proliferation and invasiveness of oncogenic cells [4, 12-14]. Hsp70 inhibits TNF-α mediated-apoptotic cell death and acts as a cell-surviving factor in hostile tumour environments. It promotes tumorigenesis and tumour development potential in cancer cells through escape immunology mechanisms [4, 12-14]. Additionally, it increases aberrant cell survival while inhibiting the activity of apoptotic protease activating factor 1 (Apaf-1) and indirectly reduces the activation of procaspase and apoptosis [4, 15]. Also known as Hsp70L1 (HSPA1A), Hsp70.14 is a variant of Hsp70 that lacks the C-terminal domain but contains the ATPase and substrate-binding domain [16, 17]. Although little is known about its functionality, it has been suggested to perform a function like Hsp70 molecules when expressed in the same mitochondrial compartment of trypanosomatid bacterial species [18]. However, Hs70L1 exhibits functional differences when interacting with antigen-presenting cells and dendritic cells to activate cytokines [19].

Considering that hostile tumour environments amass misfolded proteins, express varying levels of SNRPG, and at the same time overexpress Hsp70, these two proteins have become indispensable anti-cancer drug tools in the drug discovery parlance [9-12]. They are active in critical biological pathways and are frequently overexpressed in various cancer types, suggesting their critical roles in signal transduction and active involvement in protein

quality control systems. Their functions are predominantly mediated by PPIs, making them therapeutically vulnerable for smart drug discovery. However, these suggested PPIs remain overlooked, elusive, and yet to be characterized. Therefore, understanding their binding affinity will provide novel therapeutic insights into the development of anticancer drugs. In this study, we characterized the binding affinity, binding stoichiometry, and interaction thermodynamics between the SNRPG and Hsp70.14 oncogenic proteins using the first-ever developed MST-based assay.

Experimental methods

Expression and purification of the SNRPG and Hsp70.14 proteins

The Hsp70.14 (UniProtKB-P0DMV8 (HS71A_HUMAN)) and SNRPG (UniProtKB-P62308 (RUXG_HUMAN)) codon-optimized DNA sequences were cloned into the pGEX-6P-2 and pQE30 expression vectors respectively and purchased from GenScript, (New Jersey, USA). Amplification of the genes was done by incorporating upstream *BamHI* and downstream *XhoI* restriction enzymes. Using the *E. coli* BL21 StarTMpLysS (DE3) (Stratagene): Fomp T hsdSB (rB-mB -) gal dcm rne131 (DE3) strain, the proteins were then successfully expressed overnight in enriched media at 25°C, induced with 0.5 mM isopropyl 1-thio-β-D-galactopyranoside (IPTG) concentration. Purification of the recombinant SNRPG protein was done using a Nickel-NTA cobalt-recharged column, while Hsp70.14 was purified using a GST-agarose (SIGMA® Aldrich) column connected to an Econo® Chromatography Column (Amersham Pharmacia). Protein concentration was determined by use of a NanoDrop® ND2000 spectrophotometer (Thermo Fisher Scientific) and the elutes were then subjected to microscale thermophoresis.

Microscale thermophoresis measurement

To label a 100 µL of 100 nM 6X His-tag SNRPG protein, 0.05% Tween-20 was first mixed with 1X phosphate-buffered saline (pH 7.4) (PBS-T). Then 100 µL of the Monolith His-tag Labeling Kit RED-tris-NTA 2nd Generation (MO-L018) at a 100 nM concentration was added (NanoTemper Technologies, Munich, Germany). A final concentration of 50 nM was achieved by diluting the mixture in 1X PBS-T buffer followed by incubation in a dark room for 30 mins. This was done to obtain fluorescence signals of the SNRPG proteins that were similar to and above the Monolith NT.115 instrument's typical detection limit (NanoTemper Technologies, Munich, Germany).

Figure 3 depicts the Microscale thermophoresis studies that were carried out on a NanoTemper® Monolith NT.115 (NanoTemper Technologies GmbH, Munich, Germany). After sample preparation, premium treated capillaries were loaded with the mixture. A Titration against the 50 nM fluorescent 6X His-tag~SNRPG was accomplished using a 16-tube serial dilution of the non-fluorescent GST~Hsp70.14 protein ranging from 510 nM to 3.11×10^{-5} µM. A final volume of 20 µL per sample was mixed and added to each tube using low-bind pipette tips. After preparation of the serial dilutions, the capillaries were filled with 4 µL samples through capillary action. MST measurements were performed in triplicates within the premium capillaries (MO-K025) on the NanoTemper® Monolith NT.115 (NanoTemper Technologies GmbH, Munich, Germany). A temperature of 24.5°C, 40% MST power, and 40% light-emitting diode (LED) power were used, while laser off/on times periods of 5 seconds and 30 seconds, were employed respectively. The latest version of the MO control software (v1.6) was used to operate the system, while the NanoTemper® analysis software settings, which were used for data analysis, were optimized for TRIC-sensitive dyes.

Results

Using the RED-tris-NTA 2nd Generation dye, fluorescent labeling of the 6X His-tag SNRPG protein revealed a high affinity of 3.8 ± 0.5 nM. This was to be expected as binding amplitudes and signal-to-noise ratios are usually higher using the Monolith His-Tag labeling Kit (MO-L018) [21, 22]. **Figure 4A** shows a flawlessly overlayed graph created from MST capillary scans for the 6X His-tag SNRPG protein, suggesting that no protein was adsorbed onto the capillaries. **Figure 4B** shows the capillary scan output, confirming that there were no protein fluorescence fluctuations and the 6X His-tag SNRPG protein was successfully labelled. This is an important procedure as the adsorption of

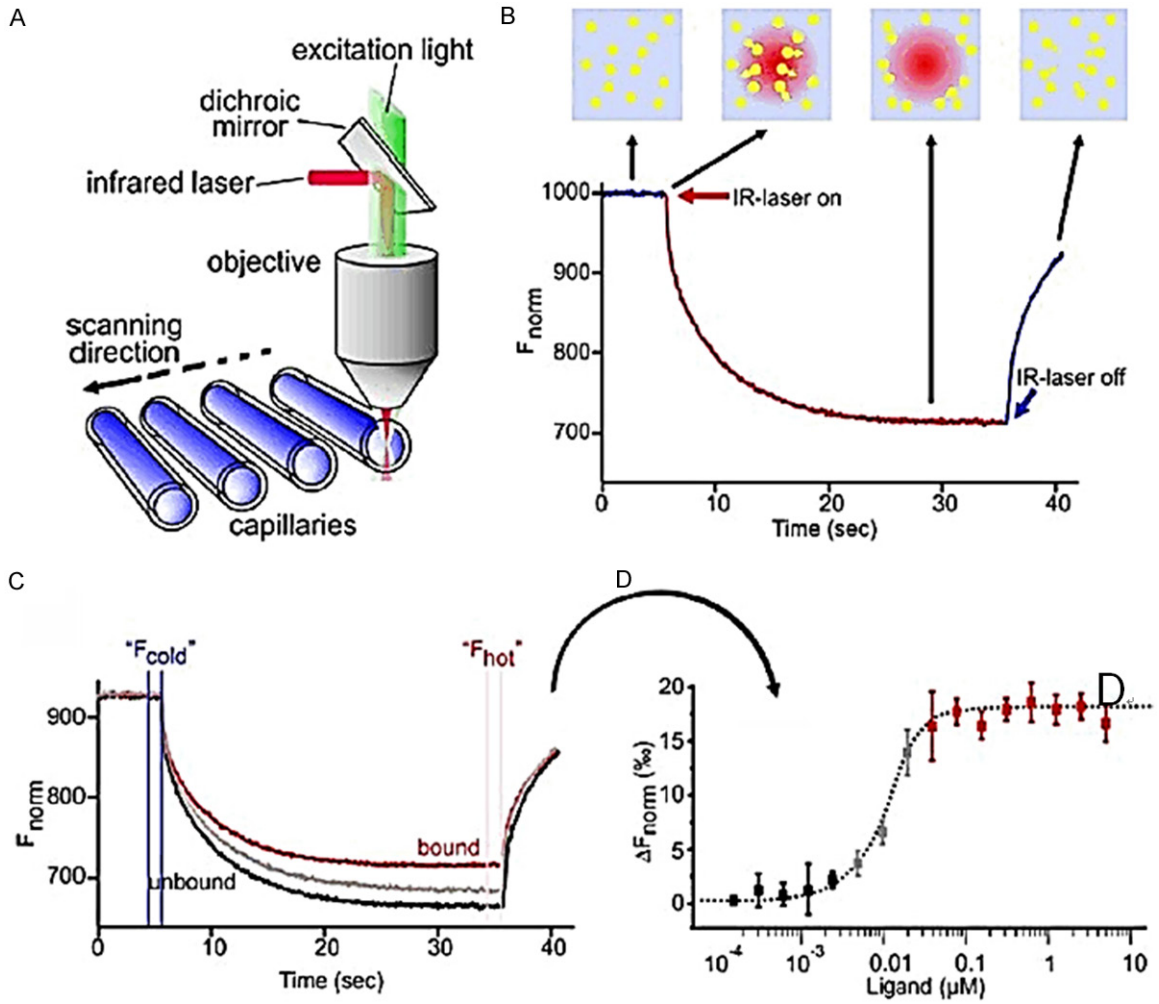

Figure 3. Setup for MicroScale Thermophoresis (MST) (A) MST tests are carried out in microscopic glass capillaries. The MST effect is triggered by infrared and fluorescence lasers, which generate sample tracking (B) The time-dependent change in fluorescence after infrared heating of the sample capillaries is explained by temperature-related intensity change (TRIC) and thermophoresis (C) For varied combination ratios of target and ligand molecules, several MST traces are acquired (D) The steady-state affinity of the target-ligand interaction can be determined through dose-response analysis of MST traces. (Figure extracted from Mabonga et al [20]).

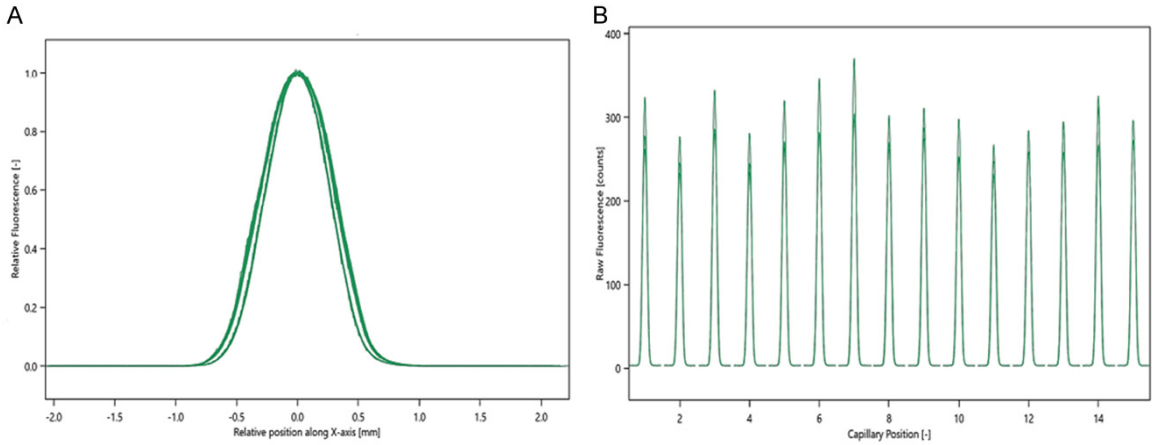

Figure 4. MST scans for the 6XHis-tag SNRPG protein in capillaries. The capillary scan graph was perfectly overlayed, indicating that there was no SNRPG protein adsorption onto the capillaries (A). As expected, the capillary scans revealed no changes in protein fluorescence, indicating that the SNRPG protein was successfully labelled (B).

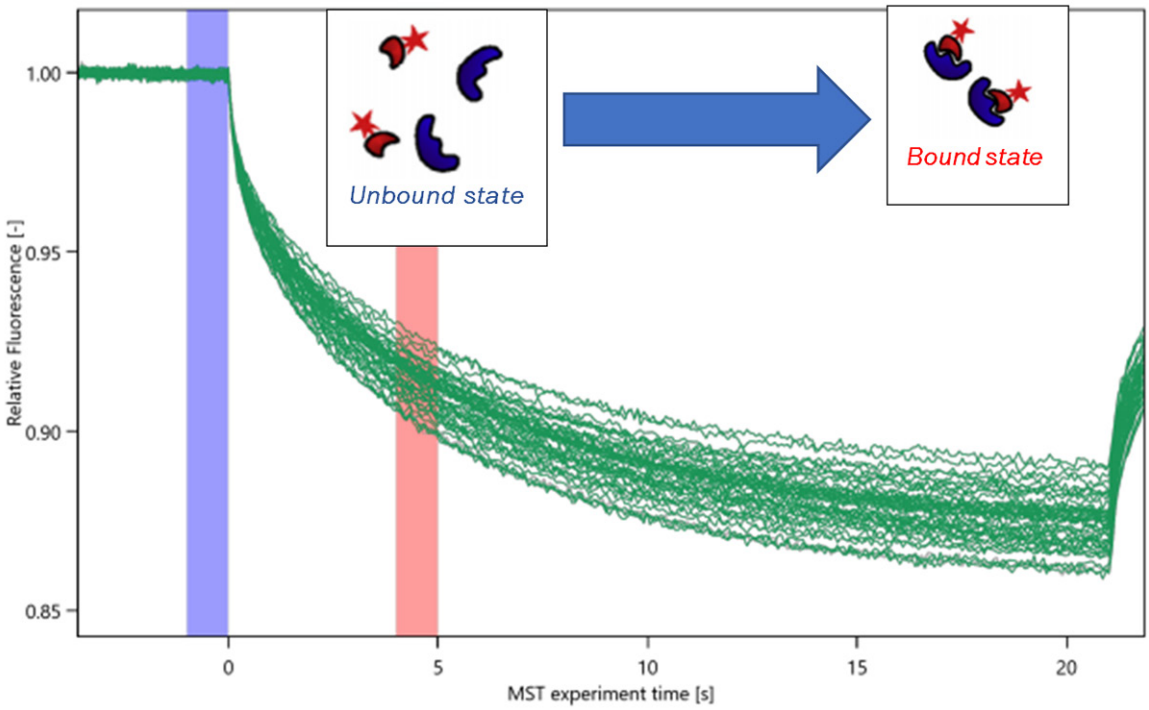

Figure 5. Thermograph of SNRPG binding to the Hsp70.14 at 24.5 °C. For varying combination ratios of the SNRPG and Hsp70.14, several MST traces were recorded. To determine the K_D of the contact and avoid potential convection phenomena, the cold zone is set to 0 seconds (blue) and the hot region to 20 seconds (red).

reference ligands and proteins onto the capillary walls subsequently results in a loss of material, which may, in turn, cause a decrease or ablation in ligand binding, thereby affecting the MST signal and consequent results [21-23].

Pre-test binding checks to ascertain detectable binding between the 6X His-tag SNRPG and the GST-Hsp70.14 proteins ensued after successful fluorescence labelling of the 6X His-tag SNRPG protein. Positive results were observed suggesting detectable binding between the two macromolecules. Pre-testing for the adjustment of concentrations or labelling is highly recommended to minimize material waste from experiments that have either failed or demonstrate indeterminant binding affinity caused by insufficient fluorescence [21-23].

MO Affinity Analysis software v2.3 was used to characterize and analyse the binding event between the SNRPG and Hsp70.14 proteins. Analysis was conducted using an MST on-time of 1.5 seconds, which was also employed for calculating the K_D value, where n = 3 independent measurements (error bars represent the standard deviation). Table S1 summaries the MST raw dataset of the merged PPI dose-response between SNRPG and Hsp70.14, while any other additional MST raw data is provided as Supplementary Data (S1). Dose-response curves were fitted to a one-site binding model to extract K_D values from a K_D-binding model assuming a 1:1 binding stoichiometry.

As is seen on the raw MST trace plot, the relative and significant changes observed in fluorescence were directly proportional to an increase in the titration concentration of Hsp70.14. The thermograph depicted in **Figure 5** shows no signs of aggregation or adhesion. This indicates the transitioning process between the two proteins in an unbound to a bound state.

The accuracy of a binding curve is highly dependent on the occurrence of three significant points that do not imply binding, the slope of binding and binding to completion [23]. A tritration curve with a conventional sigmoidal shape and a peak in the thermophoresis signal near to the apparent saturation point is shown in **Figure 6**. The findings suggest that during bind-

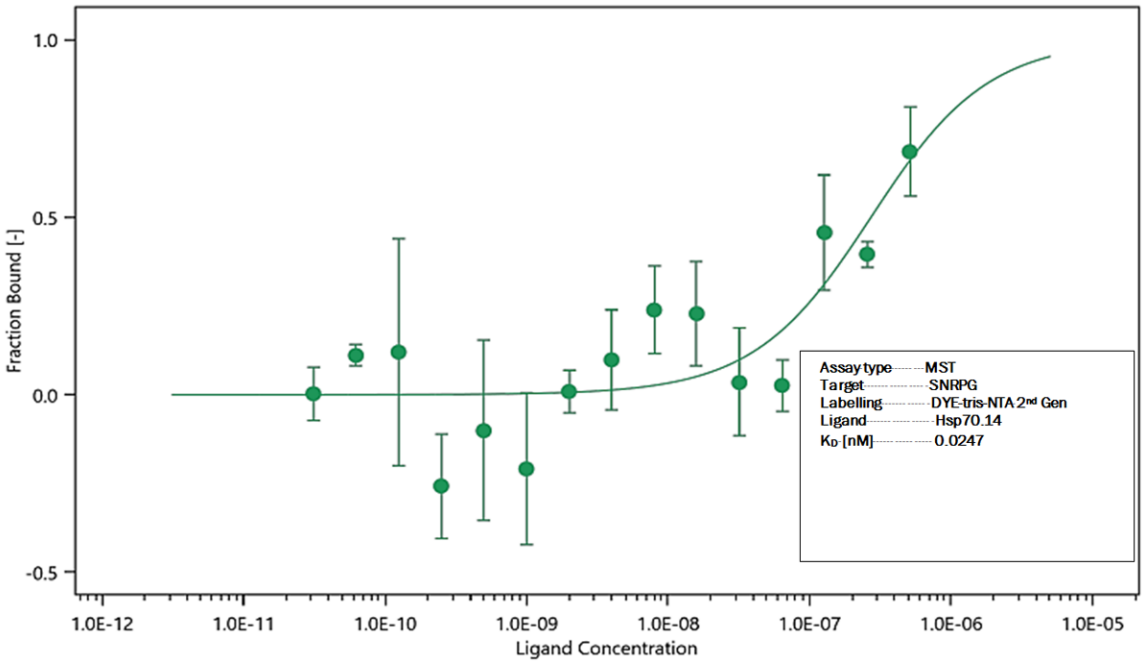

Figure 6. The binding interaction between SNRPG and Hsp70.14 has a dose-response curve. SNRPG protein concentration was held constant at 50 nM, whereas ligand concentrations ranged from 510 nM to 3.11 × 10^{-5} M. The interaction's binding affinity measurement yielded a K_D of 0.0247 nM. The studies were carried out at 24.5°C for 30 minutes at medium MST and 40 percent LED power.

Table 1. Overview of the MST dataset between the SNRPG and Hsp70.14

Description	Experiment
Target Name	SNRPG
Target Concentration	50 nM
Ligand Name	Hsp70.14
Ligand Concentration	510 nM to 3.11 × 10^{-5} μM
N	3
Excitation Power	40%
MST Power	40%
Temperature	24.5°C
K_D	2.4673 × 10^{-7}
K_D Confidence	± 3.606 × 10^{-7}
Response Amplitude	18.759832
Target Concentration	5 × 10^{-8} [Fixed]
Unbound	911.77
Bound	930.53
Std. Error of Regression	2.9145883
Reduced χ^2	2.8785179
Signal to Noise	6.9522461

ing, identical fluorescence changes and differing thermophoretic characteristics were produced. Measurements of the binding affinity under aqueous conditions between the SNRPG and Hsp70.14 proteins were determined and a K_D value of 0.0247 nM was observed (**Table 1**).

Discussion

Protein quality control (PQC) machineries play critical roles to ensure protein homeostasis in both healthy and diseased cells [24]. Targeting PPIs linked to PQC pathways is an inevitable strategy in PPI-focused drug discovery [24-26]. The demanding task for PQC machineries in hostile tumour microenvironments has furnished scientists with an ever-growing panorama of mechanistic shrewdness into pathogenesis and disease progression. PQC machinery modulate general protein turnover as well as the degradation of tumor-promoting and/or suppressive proteins, which are crucial processes in carcinogenesis and tumor growth [24, 25]. The pathogenic function of these machineries may possibly extrapolate to highly prolonged revocations and curative therapies for many incurable diseases such as cancer [25]. The pathologies associated with PPIs in malfunctioning PQC machineries and deregulation of protein homeostasis may enable the

design of specific pharmacological treatment of cancers [4]. The approach has been successful for multiple myeloma, leading to the creation of bortezomib, a proteasome inhibitor. Several other compounds have also entered the market with some currently being tested clinically and pre-clinically [27].

The rational optimization of molecular interactions between SNRPG and Hsp70.14 has been an overlooked anti-cancer strategy for many years. Deciphering the modus operandi between the two proteins might reap enormous therapeutic benefits in the cancer world. A vast range of biological activities link the two proteins, and they play crucial roles in cancer-cell processes [8, 15]. As precursors to major and minor spiceosomes, small nuclear ribonucleoproteins (snRNPs), require SNRPG for their formation [28-31]. The proteostatic regulation of SNRPG by PQC machineries is of particular interest in hostile tumour microenvironments [5]. Regulation of SNRPG by chaperone-mediated autophagy (CMA) is supposedly modulated by Hsp70 among other PQC substrates [14, 15]. However, the precise mechanism of involvement between SNRPG and Hsp70 in both healthy and hostile tumour microenvironments remain elusive and yet to be characterised.

This study provides the first line of evidence in quantifying the interactive affinity between SNRPG and Hsp70.14 using the MST assay. The results from the MST experiment between SNRPG and Hsp70.14 observed high binding affinity with a K_D value of 2.4673×10^{-7} (**Table 1**). The experiment was conducted at medium MST and 40% LED power with 30 minutes incubation time, at 25°C. The results provide the first optimized methodology suitable for studying PPIs between oncoproteins involved in protein homeostasis in PQC machineries. The strong binding between the SNRPG and Hsp70.14 proteins suggests possible involvement between the core splicing protein and molecular chaperone as shown by the K_D value for the low nanomolar concentration range. In pharmacological research, this observation is particularly interesting because it provides first stage biological insights into the possible involvement between the two proteins in hostile tumour microenvironments. The thermatic interaction provides a critical therapeutic foothold into the mechanistic structure-function relationship towards the development of anti-cancer drugs [8].

With the realization that most PPI-focused anti-cancer drug targets are poorly suited for engagement by orthodox large molecule drug classes, small molecule inhibitors have provided powerful impetus as new structural classes of drugs that could fill the existing interludes in the drug discovery parlance [32-37]. The design of small molecule drugs is an indispensable smart drug tool to modulate the weak and therapeutically vulnerable SNRPG~Hsp70.14 interaction. According to Salahudeen and Nishtala [38] PPIs with low nanomolar concentrations are critical in modulating cancer-relevant biological processes and have prompted scientists to develop architecturally complex synthetic mimicries as anti-cancer inhibitor drugs.

Generally, lower K_D values resemble stronger binding and higher binding affinity. Conversely, weaker and lower binding affinities are associated with higher K_D values. From a drug discovery perspective, weak PPIs with lower K_D values (i.e., high binding affinity) are therapeutically vulnerable as drug targets [38, 39]. Such PPIs have a low binding affinity and can be inhibited with small molecules in PPI-focused drug development [38]. Thus, the weak SNRPG~Hsp70.14 interaction can be a good starting point to perform high-throughput screening of small molecule 'lead compounds' as anti-cancer drug candidates. The modulation of the SNRPG~Hsp70.14 interaction may represent a breakthrough in the biomedical field [8, 40, 41].

The activity of a slew of PPIs is activated when autophagy is modulated in PQC between signalling and regulatory molecules [42, 43]. SNRPG~Hsp70.14 interaction has clinical importance as well as prognostic consequences in tumorigenesis and tumour development, but this remains a matter of debate. However, suggestive evidence indicates that SNRPG~Hsp70.14 interaction may represent key processes in tumour initiation, progression, and resistance to cancer therapies [8, 41]. The interaction may intriguingly emerge as a critical arsenal in patient stratifications as well as a conjecturing prognostic biomarker. Thus, strategies to inhibit the SNRPG~Hsp70.14 interaction may accelerate the emergence of next-generation pharmacological drugs targeting the

PQC as new tools for anticancer therapies [8, 40].

Targeting the SNRPG~Hsp70.14 interaction in the autophagy PQC pathway is ultimately a new anti-cancer therapeutic option. The uncertain link between SNRPG and Hsp70 in cancer development provides an opportunity to target the regulation of autophagy PQC machinery as a PPI-focussed anticancer therapy at various stages of cancer. Targeting autophagic PQC pathways is a promising approach to perambulate outcomes in cancer treatment. More generally, it is suggested that an autophagy defect leading to the dysregulation of proteins like SNRPG would facilitate tumorigenesis and tumour development [5, 8, 42, 43]. The up-regulation of Hsp70 in autophagic PQC pathways may compensate the amassed SNRPG proteins in growing tumours and may help to combat hostile tumour microenvironments [8, 14]. Thus, the successful characterization of SNRPG~Hsp70.14 PPI may present an inevitable strategy to develop anti-cancer drugs.

Over the years, successes in targeting PPIs linked to autophagic PQC pathways as potential drug targets have been reported. Many anticancer drugs targeting molecular chaperones are already on the market with some having already entered the clinical setting [23]. Examples of drug agents linked to PQC mechanisms are highlighted in **Table 2**. However, PPI-focused smart drug molecules linked to the interaction between SNRPG and Hsp70.14 have not yet been identified or characterised. Identifying small molecule inhibitors as drug leads for the SNRPG~Hsp70.14 interaction may possibly usher the final pinnacle of human ingenuity in alleviating cancer challenges [8].

Targeting PPIs between regulatory proteins linked to PQC in developing anti-cancer drugs remains the quintessence of the future of PPI-focused drug discovery. Given the importance of protein homeostasis in biological systems and its complexity, targeting the interaction between SNRPG and Hsp70.14 for drug discovery in cancer may provide new perspectives in PPI-centred drug discovery. The biological connection between the molecular chaperone Hsp70 and the splicing protein SNRPG remain an overlooked arsenal in the design and development of anticancer therapies. Defects in SNRPG regulation have been linked to abnormal splicing patterns in a variety of apoptotic factors, resulting in tumourigenesis and tumor growth [5, 8]. Therefore, PPIs between anti-apoptotic and proapoptotic proteins linked to the PQC may pave the way for the creation of novel anti-cancer drugs [5, 8, 49]. Thus, targeting the interaction between SNRPG and Hsp70 in PPI-focused drug discovery may represent the final panorama of human ingenuity in alleviating the modern-day cancer challenges.

Conclusion

Based on growing research findings and clinical trials, it is beyond doubt that PQC and protein homeostasis pathways are critical for cancer cell survival. Targeting these pathways not only aids drug discovery in the biopharmaceutical world, but it also aids in answering fundamental concerns about cancer biology. To this end, novel proteins that regulate proteostasis pathways and the splicing machinery, such as the Hsp70.14 and SNRPG are currently being evaluated for PPI-focused anti-cancer drug discovery.

Potent and authentic determination of the binding affinity between SNRPG and the Hsp70.14 is crucial in deciphering the relationship between the PQC and splicing machineries in both health and hostile tumor microenvironments. Quantitative analysis of the intermolecular interaction between the two regulating oncogenic proteins is critical for the development of innovative and effective anti-cancer therapies. Analysis of the MST results demonstrated in the present study is the first *in vitro* mechanistic elucidation into the interaction between SNRPG and Hsp70.14

The study affirms the overlooked scientific pursuit insinuating the possibility of *in vivo* involvement of two regulatory proteins, and strongly establishes possible involvement between SNRPG and the Hsp70.14 in cancer-cell networks. Furthermore, it disqualifies the oversight that has been placed on the prospective use of the SNRPG~Hsp70.14 interaction in developing PPI-focused smart drugs. Extrapolated exploration of the molecular and structural interplay between the two proteins may yield to curative therapies. Thus, small molecule 'lead' compounds capable of modulating the interaction may lead to the discovery, design and development of possible anti-cancer nootropics.

Table 2. Pre-Clinical/Clinical Drug Agents Targeting the Protein Quality Control Mechanisms

Pathways	Compounds	Target	Drug development stage
Unfolded protein response	STF-083010	Inhibitor of XBP1 splicing [44]	Pre-clinical (in vivo)
	Salicyaldehyd	Inhibitor of the IRE1 αendoribonuclease activity [45]	Pre-clinical (in vivo)
	MKC-3946	Inhibitor of XBP1 splicing [46]	Pre-clinical (in vivo)
	Toyocamycin	Inhibitor of XBP1 splicing [47]	Pre-clinical (in vivo)
	GSK265615	Inhibitor of PERK phosphorylation [48]	Pre-clinical (in vivo)
	GSK260641	Inhibitor of PERK phosphorylation [48]	Pre-clinical (in vivo)
	ISRIB	Activator of eIF2β [49]	Pre-clinical (in vivo)
	Salubrinal	Inhibitor of GADD34 [50]	Pre-clinical (in vivo)
	Guanabenz	Disruptor GADD34-PP1c complex [51]	FDA approved (HT), first approved in 1994
	Compound 132	Selective activator of ATF6 [52]	Pre-clinical (in vivo)
	Compound 263	Selective activator of ATF6 [52]	Pre-clinical (in vivo)
	Ceapins A7	Inhibitor of ATF6α [53]	Pre-clinical (in vivo)
Heat shock proteins	Geldanamycin	Inhibitor of Hsp90 [54]	Phase I clinical trial
	17-AAG	Inhibitor of Hsp90 [55]	Multiple Phase I clinical trials
	Alvespimycin	Oral inhibitor of HSP90 [56]	Multiple Phase I/II clinical trials
	Retaspimycin	ATPase inhibitor of HSP90 [56]	Multiple Phase I/II clinical trials
	Luminespib	Second generation HSP90 inhibitor [56]	Multiple Phase I/II clinical trials
	Onalespib	Selective HSP90 inhibitor [56]	Multiple Phase I/II clinical trials
	Ganestespib	Potent inhibitor of HSP90 [56]	Multiple Phase I/II clinical trials
	Triptolide	HSF1 transactivation domain inhibitor [57]	Multiple Phase I/II/III clinical trials
	Rohinitib	HSF1- DNA binding inhibitor [58]	Pre-clinical (in vivo)
Autophagy	Rapamycin	Allosteric inhibitor of mTOR1 [59]	FDA approved (IS), first approved in 1999
	Temsirolimus	Specific inhibitor of mTOR [59]	FDA approved (RCC), first approved in 2007
	Everolimus	Selective mTORC1 inhibitor [59]	FDA approved (RCC), first approved in 2009
	ACY-1215	Potent and selective HDAC6 inhibitor [60]	Multiple Phase I/II clinical trials
	ACY-241	Potent and selective HDAC6 inhibitor [61]	Multiple Phase I clinical trials

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Disclosure of conflict of interest

None.

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Table S1. MST raw data of merged dose-response of the PPI between SNRPG and Hsp70.14

Dose	Response (Average)	Std. Dev.	N
5.1E-07	924.61335	2.37565	3
2.55E-07	919.19981	0.67155	3
1.275E-07	920.33991	3.01888	3
6.375E-08	912.26622	1.33844	3
3.1875E-08	912.41764	2.84477	3
1.59375E-08	916.05525	2.77107	3
7.96875E-09	916.24642	2.2942	3
3.984375E-09	913.61667	2.67776	3
1.992188E-09	911.94189	1.09236	3
9.96094E-10	907.85358	4.03257	3
4.98047E-10	909.8712	4.77011	2
2.49023E-10	906.94954	2.77234	3
1.24512E-10	914.02444	6.01046	3
6.2256E-11	913.84861	0.56001	3
3.1128E-11	911.82478	1.39014	2
1.5564E-11	Outlier	Outlier	Outlier