

## Molecular Chaperones and Co-chaperones as Therapeutic Targets for Cancer

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### Introduction

Cancer is a devastating disease that has affected millions of people, consumed tremendous efforts in the treatment/care and is incurable. Statistics from [www.seer.cancer.gov](http://www.seer.cancer.gov) show 14,140,254 people were living with cancer in 2013 in US with 1,685,210 new cases projected and 595,690 estimated deaths to occur due to cancer in 2016. Number of people surviving cancer (measured as 5-year survival rate) has increased by 18% (48.7% in 1975 to 66.9% in 2012) during the last half century. This number appears to be small if we consider the contribution of proactive care for example, early detections, and attention to life style and aggressive chemical/radiation therapy and at the same time stare at a bleak hope of any apparent breakthrough in next 10 years. On the other hand, gain of even a small percentage in the survival of cancer can be regarded significant considering its complexity in terms of causes, types, organs affected, severity, origins and metastasis each of which has made it very difficult to combat this disease (system) [1]. For the sake of comparison, unlike AIDS, cancer is not limited to a pathogen and immune system only and unlike Alzheimer's or Parkinson's it is not restricted to one proteins and one organ only. This is not to say other diseases are less complex but certainly not as diverse as cancer. Hence, knowing well the challenges in dealing with an intricate system and to develop a cure, the need for extensive investigation in the area of cancer is imperative.

### Drug Design for Cancer

Keeping the transcriptome (RNA) influence aside because of its infancy in the field, we can generalize that: oncogenic stimuli by an extrinsic carcinogen or intrinsic oncogene (proto oncogene/genetic manipulation) gets transcribed in genome and manifests itself through proteome. Literature is filled with examples of protein kinases and signal transducing protein molecules, under oncogenic conditions, playing a pivotal role in the transformation of normal tissue leading to poor prognosis. And precisely for this reason, proteins have been used as targets for drug design against cancer and sometimes with good success. One such textbook example is provided by drug design against bcr-abl kinase that causes chronic myelogenous Leukemia (CML). Tireless efforts by dedicated scientists resulted in the design of a molecule 'imatinib' that inhibited bcr-abl kinase reducing the annual CML relapse to 0.6% [2]. A careful analysis of relapsed tumors indicated generation of mutated forms of bcr-abl kinase that were refractive to imatinib inhibition. But diligent investigations lead to the characterization of each bcr-abl mutant followed by modifications in the design of original imatinib. This approach produced new inhibitors of bcr-abl for patients with relapsed CML. Even the most resistant mutants (T315I) were successfully inhibited by clever drug (AP24532)

design [3]. These examples therefore provide inspiration for discovery of new protein targets in cancer and subsequent drug design against them for a cure. Chaperones are a group of protein molecules that have been vigorously targeted for cancer therapeutic development during the last two decades [4].

### Molecular Chaperones

Chaperones were identified around 1962 as proteins produced by heat shock in *Drosophilla* [5] and soon a big family of these molecules was discovered. They were thus called Heat Shock Proteins (HSP) and differentiated on the basis of their molecular weight. For example, HSP60 is a 60 kDa chaperone molecule. The name "chaperone" was proposed by Ellis based on the protective nature of these proteins [6]. For simplicity, recent classification has grouped HSP molecules into different families viz., Hsp90 (HSPC), Hsp70 (HSPA), Hsp60 (HSPD), small Hsp (HSPB) and large Hsp (HSPH) [7]. Normal function of chaperones is to fold nascent polypeptide chains or unfolded proteins into their active conformation, [8,9]. They achieve this at the expense of ATP hydrolysis. Each family is further composed of additional members that help in carrying out concerted steps meticulously [10]. These supporting proteins are called "co-chaperones." Co-chaperones and many chaperones lack the ability to hydrolyze ATP and are unable to fold protein on their own but at the same time they may bind to client proteins and prevent unwanted aggregation or toxicity. In such cases they are referred to as 'holdases' [11].

Molecular chaperones were identified as anti-cancer drugs since early 1990s [12]. We will discuss anticancer drugs designed using Hsp90 and Hsp70 as examples.

### Hsp90

Hsp90 is a dimeric protein with each monomer consisting of three domains; i) top nucleotide binding domain [(NTD) binds ATP], ii) middle domain [(MD) binds substrate protein] and iii) bottom c-terminal domain [(CTD) responsible for dimerization]. Hsp90 is highly interactive protein exhibiting a huge list of protein partners (<https://www.picard.ch/Hsp90Int/index.php>) that makes it difficult to provide a concise mechanism. A typical Hsp90 cycle however involves binding of the substrate (unfolded protein) either free or handed over by Hsp70 system mainly to MD while NTD is in ATP state [13]. Subsequent binding by co-chaperones like p23, Sba1, Cdc37, p50 or similar others stabilize the complex either by inhibiting ATP hydrolysis or enhancing 90-substrate interactions. This allows substrate a chance to regain physiological folding. Proteins like Aha1 then trigger ATP hydrolysis and disintegration of complex leads to release of folded

substrate. Hsp90 dimer still connected by CTD is ready to take new ATP and undergo a fresh cycle. The folding cycle involves contribution of many other co-chaperones that simply cannot be discussed here [14].

Inhibition of Hsp90 causes accumulation of mis-folded oncogenic proteins [15]. Hsp90 thus was one of the first chaperone targets used for anti-cancer drug design [12]. Since then it has progressed further and Hsp90 has recently been named an unlikely ally in the war on cancer [16,17]. Its co-chaperones are equally deemed as targets. P23 is among many of Hsp90 co-chaperone that is overexpressed in breast cancer [18], is involved in prostate cancer through androgen receptor activity [19] and is overexpressed in acute lymphoblastic leukemia where it inhibits chemotherapy-induced apoptosis [20].

Since the first Hsp90 inhibitors geldanamycin and radicicol exhibited anti-cancer property development of more drugs in this direction have been designed [4,12]. More soluble and less toxic compounds like 17-AAG, KOS-953, have shown promise in cancer treatment [21]. Celestrol interferes with Hsp90/Cdc37 complex inhibiting growth-regulating pathway and is considered a promising candidate for prostate cancer treatment [22]. Drug 'gedunin' binds to P23 and restores the apoptotic pathways of malignant cells [23]. Drugs like Retaspimycin (IPI-504), Ganetespib (STA-9090) are candidates in the on going phase 1-3 clinical trials targeting Hsp 90 in various cancers [24].

## Hsp70

Hsp70 is the main workhorse of folding machinery in humans that helps nascent or unfolded proteins to fold into biologically relevant structure. In Hsp70 folding cycle first a client protein, either itself or facilitated by Hsp40, binds to Hsp70 C-terminal substrate binding domain (SBD) with ATP bound in its N-terminal Nucleotide binding domain (NBD). A substrate/Hsp70/Hsp40 ternary complex is thus formed [10]. Formation of this complex is accompanied by hydrolysis of ATP into ADP. In the next step nucleotide exchange factors (NEF) like (GrpE or Bag or similar proteins) induces exchange of ADP with ATP in the NBD [25]. This step is associated with the release of substrate possibly as folded active protein. If the substrate fails to fold it enters Hsp70 cycle many times until it is successful otherwise it gets tagged for degradation through CHIP -ubiquitin pathway. The system also participates in apoptosis [26]. While Hsp70 and its co-chaperones are believed to act in concerted fashion we have shown the individual members can have differing effects on the substrate molecule [27]. Hsp70 cycle thus aimed to maintain homeostasis and the quality of protein inadvertently or under duress helps healthy folding of oncogenic proteome facilitating tumor genesis [28].

The first indication of Hsp70 involvement in cancer comes from its overexpression in tumors [29-31]. Sometimes levels of chaperone transcription factor (HSF1) are directly related to the severity of cancer [32-34]. In addition, Hsp70 suppresses tumor through senescence pathways [35]. Hsp70, Hsp70.2 and mitochondrial Hsp70 when inhibited induce apoptosis in breast cancer cells [36]. Over expression of Hsp70 was found to be responsible for resistance to cell death in pancreatic cancer [37]. Metastatic Hepatocellular Carcinoma cell lines have been reported to exhibit higher levels of Mortalin (mitochondrial Hsp70) and Mortalin-mRNA [38].

Our NMR work in recently published article showed development of two drug like molecules i) Telmisartin that disrupts Hsp70/GrpE interaction and Zafirlukast that disrupts Hsp70/Hsp40 interaction

[39]. In another recent article again our NMR studies showed development of a modified form of MKT-007, an anti-cancer drug, that was 3-fold more active than original MKT-007 on breast cancer cell lines MDA-MB-231 and MCF-7 with biological half-life in microsomes improved 7-fold over the original compound [40]. Thus, these initial findings clearly indicate there is potential for anti-cancer drug development targeting Hsp70 system and systematic investigations in this direction could be highly applicable in finding cancer cure. Further, other chaperones besides Hsp70 and 90 have also been targeted for drug design [41,42].

In conclusion, chaperones appear to cross the pathway of a transforming cell at many levels and in many forms and some of their inhibitors have advanced in clinically trials. These facts are convincing reasons to pursue rational drug design using chaperones as anticancer therapeutic targets.

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