

Targeting the SUMO E2 Conjugating Enzyme Ubc9 Interaction for Anti-Cancer Drug Design

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Abstract: Sumoylation has been implicated in a variety of cancers, suggesting that sumoylation manipulation could be one approach for regulating tumorigenesis. Ubc9 exerts a central function for the sumoylation pathway, interacting with almost all the partners required for sumoylation. The high-resolution structure available for Ubc9 as well as the recent determination of more interacting partner complex structures makes rational drug design that target Ubc9 possible. Structure-based virtual drug screening has been used increasingly as the first step of drug design to select potential lead templates.

This review analyzes all the interfaces between Ubc9 and its binding partners while also highlighting the possible targeting sites on Ubc9 best suited for virtual screening and drug design.

Key Words: Sumoylation, Ubc9, virtual screen, structure.

INTRODUCTION

Sumoylation regulates diverse cellular functions, including DNA replication and repair, chromosome packing and dynamics, genome integrity, nuclear transport, signal transduction, and cell proliferation [1-4]. Similar to ubiquitination, sumoylation requires three steps of enzymatic reactions to attach the small ubiquitin modifier (SUMO) protein to the substrates. Each step requires a set of enzymes to assist the reaction, including an activating enzyme E1, a conjugating enzyme E2, and a ligase E3. For ubiquitination, approximately 10 of E1, 100 of E2, and 1000 of E3 have been identified that assist with the enzymatic reactions. However, there are far fewer enzymes involved in sumoylation reactions. Ubc9 is the only known E2 conjugating enzyme that exists for sumoylation. The protein sequence of human Ubc9 is identical to the mouse's and closely related to *S. cerevisiae* (56%) and *S. pombe* (66%). Ubc9 is essential in all organisms except *S. pombe*, which shows the same phenotypes as SUMO and E1 deletions [4].

Sumoylation has been characterized with more than 100 different targets in a variety of organisms, many of them related to tumorigenesis. SUMO E2 conjugating enzyme Ubc9 was found to be highly expressed in many types of human cancer cells. For example, Ubc9 was highly expressed in lung adenocarcinoma [5, 6], melanoma-infiltrated lymph nodes [7], and prostate cancer cells [8]. In addition, the increased expression has been reported in several human ovarian cancer cell lines such as PA-1 and OVCAR-8 as well as in ovarian tumor tissues [9]. In addition, Ubc9 over-expression has also been linked to breast cancer development [10, 11].

Although accumulating evidence indicates that Ubc9 is upregulated in a variety of tumor cells, the mechanism of Ubc9 in cancer cells remains largely unknown. It may possibly occur by involving the process of substrate sumoylation or desumoylation. Nevertheless, the higher expression level of Ubc9 in tumor cells suggests that Ubc9 could be an attractive target for drug design by interfering with SUMO and the interacting partners, therefore potentially controlling the tumor progression. Ubc9 structures have been determined alone and in complex with SUMO, E1, E3, and the substrate, which facilitates the virtual screening of potential drugs against Ubc9. In this review, we focus on the structural aspects of

Ubc9 and its interacting partners, in particular, analyzing the interaction surface. We expect that by identifying the potential targeting sites, we will be able to use virtual screening to design small chemical compounds to manipulate tumor growth.

The Structure of Ubc9

The human Ubc9 structure has been determined by two individual groups [12, 13]. The 158 amino acid protein forms a single domain structure similar to other Ubc proteins in the ubiquitin pathway. It contains a four-stranded antiparallel β -sheet in the core and is surrounded by four helices at both ends, as shown in Fig. (1). The catalytic site Cys93, which forms a thioester bond with SUMO, is situated in the pocket between the fourth β -strand (β 4) and the second α -helix (α 2).

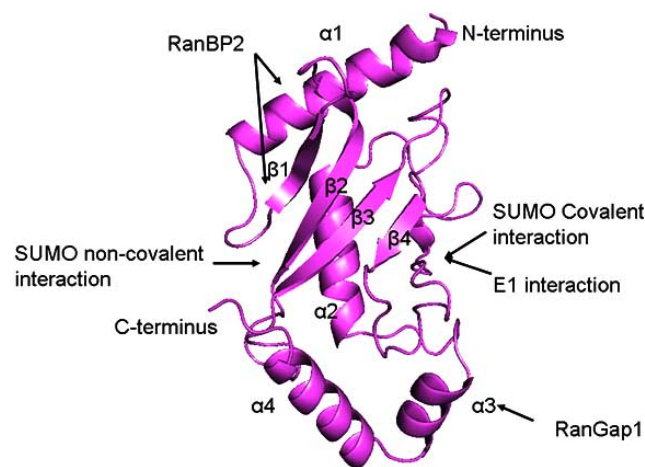


Fig. (1). Overall structure of Ubc9. Ubc9 contains a four-stranded antiparallel β -sheet in the core and is surrounded by four helices at both ends.

The surface of Ubc9 consists of a strong electrostatic dipole compared to the other ubiquitin E2 conjugating enzymes. The positive patches are scattered on one side of Ubc9, including the N-terminal region, which is composed of a region with positive and hydrophobic residues. This region is highly conserved in all the Ubc9s.

In the sumoylation process, Ubc9 functions as the core and interacts with all the other components in the modification process.

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Here, we dissect the individual interfaces and analyze the possible targeting sites for virtual drug screening and drug design.

Ubc9 with SUMO

The structures of yeast SUMO (Smt3) and human SUMO-1, 2,3 have been determined [14-18]. All of them share a compact core in their middle and an extended N-terminus and C-terminus in each direction. The contacts between Ubc9 and SUMO occur through either covalent or non-covalent interaction.

The SUMO modification is a sequential procedure. First, SUMO C-terminal Gly-Gly residues are activated and attach to the E1 conserved Cysteine to form an intermediate covalent thioester bond. This intermediate thioester bond transfers to the E2 conjugating enzyme Ubc9 at Cys 93, forming another transient intermediate bond. Therefore, the site around Cys 93 of Ubc9 is the contact region for the covalent intermediate interaction. In addition, Ubc9 interacts with SUMO through non-covalent interaction with relative high affinity ($K_d=82\text{nM}$) [19]. This interface is located on the opposite site of the Ubc9 catalytic site. For virtual screening against the interface between Ubc9 and SUMO, both the covalent interaction site close to the region near Cys 93 and the non-covalent interface are possible targets.

The detailed structure for the covalent linked SUMO to Ubc9 was deduced from SUMO-RanGap1-RanBP2-Ubc9 [20]. This structure showed an isopeptide bond formed between SUMO Gly and substrate RanGap1 Lys 524. Aside from the covalent isopeptide bond, a larger interface is observed close to the covalent interaction, including the region from SUMO Gln 92 to the end tail of Gly 97 contacting Ubc9 helix 2 and Ubc9 active site residues. For drug design purposes, the groove formed by Ubc9 residues Asn 85, Cys 93 to Ile 96, Arg 104, Gln 111, Leu 114, Gly 115, Glu 118, Glu122, Asn 124, and Asp 127 is a potential site to target as shown in Fig. (2).

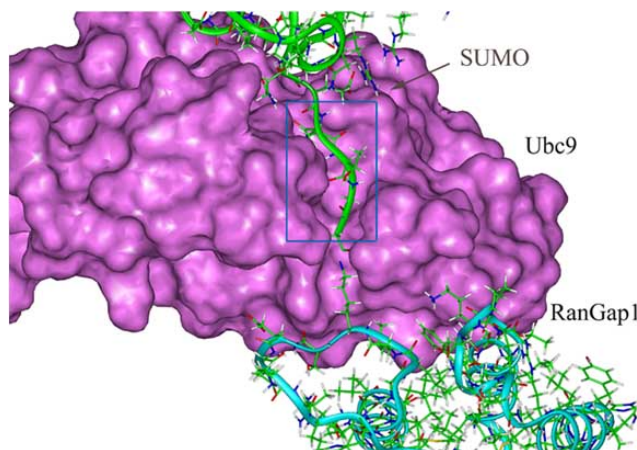


Fig. (2). The Ubc9-SUMO covalent interaction. The C-terminus of SUMO inserts into the groove of Ubc9, which is close to the Ubc9 active site Cys 93. Shown with a blue box, the groove on Ubc9 is a potential target site.

The non-covalent interaction between Ubc9 and SUMO complex structures has been determined by three groups independently [19, 21, 22]. With SUMO binding, the Ubc9 structure did not make any conformational changes. The SUMO is located on the opposite region of the Ubc9 catalytic site Cys 93. Although the N-terminus and C-terminus of Ubc9 are disordered in the structure, it is not possible that the N-terminus or C-terminus could stretch to overlap the active site. This suggests that SUMO might not interact with the same Ubc9 molecule, but rather another one in the reaction. Therefore, it could form a higher order reaction chain. For the non-covalent interaction between Ubc9 and SUMO, the residues on Ubc9 include Arg 13 through a hydrogen bond that interacts with

SUMO. The Ubc9 Arg 17 side-chain contacts the SUMO Gly 81 carbonyl oxygen and also makes two salt-bridging interactions with the side-chain from SUMO. In addition, Ubc9 Phe 22 and Gly 23 interact with SUMO through backbone atoms or through van der Waals interactions. Since the non-covalent interaction region between Ubc9 and SUMO is clustered in the pocket of Ubc9 around Arg 13 to Gly 23, the groove formed by residues Arg 17 to His 20, Phe 22, Gly 23, Tyr 25, Lys 27, and Lys 30 as shown in Fig. (3) could be used as a targeting site to interfere with the Ubc9 and SUMO non-covalent interaction.

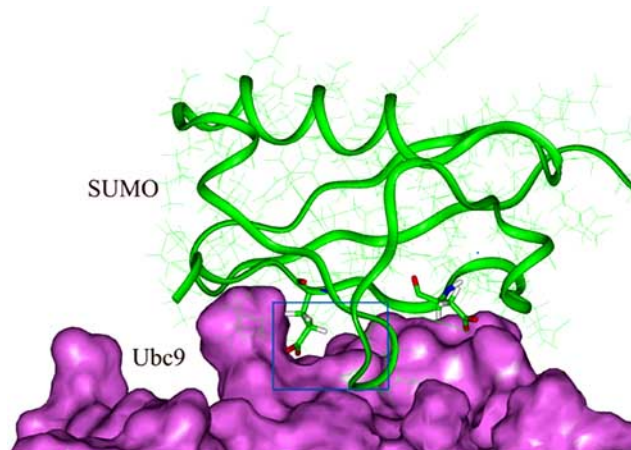


Fig. (3). Non-covalent interaction between Ubc9 and SUMO. SUMO is located on the opposite region of the Ubc9 catalytic site of Cys 93. Shown with a blue box, the area around Ubc9 Arg 17 could be targeted.

Ubc9 and E1 Interaction

SUMO E1 is a heterodimer protein that contains Aos1 and Uba2. Aos1 is a single domain protein, while Uba2 contains three domains including the catalytic cysteine domain, the adenylation domain, and the ubiquitin-fold domain. The catalytic cysteine domain has been confirmed to interact directly with Ubc9. The interaction between Ubc9 and SUMO E1 has been studied by NMR [23] with the full-length of Ubc9 and the E1 cysteine domain. In the structure, the two catalytic Cys residues, Cys 173 of E1 and Cys 93 of E2, are close to approaching each other. The distance between the two sulfurs is approximately 14 - 17Å. Since there is no protein portion in this region, the authors suggested that there might be a slight structural adjustment by binding the thioester-bonded SUMO to facilitate bond formation. Therefore, this catalytically Cys site would be a promising site to target as shown in Fig. (4).

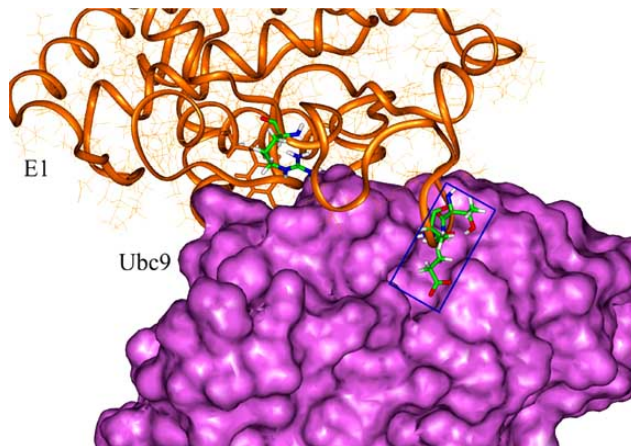


Fig. (4). Ubc9 and E1 interaction. The two catalytic Cys residues of E1 and E2 are close to each other. Shown with a blue box, the Cys 93 on Ubc9 is a good site to target.

In addition, by using a chemical disturbing method, the structure of residues 129-134 of Ubc9 have been found to have largely changed during complex formation [23]. From the structure of the complex, this region is closely located to the E1 Cysteine domain. Therefore, it also has potential as a good region for drug targeting. However, since residues 130-134 form a helix, it probably requires a peptide instead of a small molecular ligand to interfere with the interaction. From the structural observation, two loops on Ubc9 from residues 94-104 and residues 123-128 have a close interaction with E1. These loops shown in Fig. (4) are the regions that could be targeted.

The Interface Between Ubc9 and Substrates

Although numerous substrates have been found to be sumoylated, the only available complex structure for Ubc9 is from the substrate RanGTPase-activating protein 1 (RanGap1). The structure determined with the heterodimer Ubc9/RanGap1 and tetramer Ubc9-RanBP2-RanGap1-SUMO showed that the interface between Ubc9 and substrate RanGap1 is consistent [20, 24]. The binding region on Ubc9 is mainly on helix $\alpha 3$ and the residues around catalytic Cys 93. For drug targeting, Fig. (5) shows that the pocket on Ubc9 formed by residues Lys 74, Tyr 87, Ser 89, Thr 91, Cys 93, Asp 127, Pro 128, Ala 129, and Tyr 134 could be a potential site.

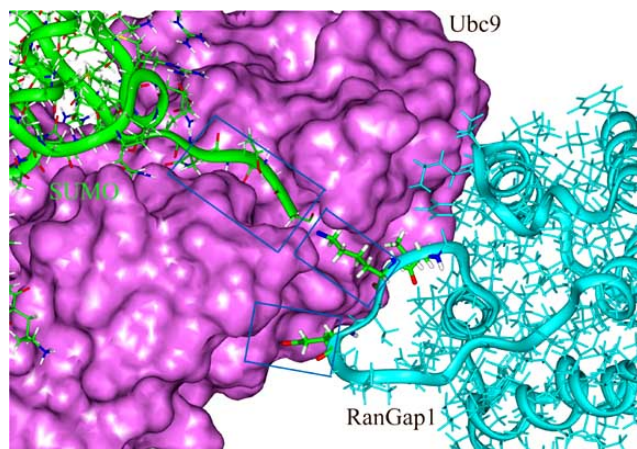


Fig. (5). Ubc9 and substrate RanGap1 interaction. The binding region for RanGap1 is mainly on Ubc9 helix 3 and residues around catalytic Cys 93, all shown with blue boxes.

However, mutagenesis on this region to test the general mechanism of Ubc9 on the other substrates, I κ B α and p53, revealed that this region has little to no effect on SUMO conjugation of these substrates, indicating that RanGap1 might interact with Ubc9 uniquely. To design a drug that specifically interferes with the sumoylation of RanGap1 through the interface between Ubc9 and RanGap1, this region has potential as a target. However, to interfere with the Ubc9 and general substrate interaction, more E2/substrate complex structures are required to find the general motifs.

Ubc9 and E3 Ligase

Three types of SUMO E3 ligase have been identified, including the SP-type, which contains SP-ring finger motif, the nucleoporin RanBP2 (Nup358), and the polycomb protein Pc2. The available Ubc9-E3 complex structure is for Ubc9-RanBP2. The interface between Ubc9 and RanBP2 was deduced from the structure of SUMO-RanGap1-Ubc9-Nup358 [20]. In this complex structure, SUMO E3 ligase RanBP2 did not interact with substrate RanGap1 directly, but had a large interface with Ubc9. The function of RanBP2 is to align the E2 Ubc9 together with the substrate RanGap1, which makes sumoylation more efficient.

The RanBP2 IR1 domain interacts with Ubc9. IR1 forms a non-global and extended structure, warping around Ubc9 with a large contact interface from the Ubc9 N-terminus to $\beta 3$ as shown in Fig. (6). Although there is a large interface between these two proteins, for the same reason, RanBP2 is a special type of E3 ligase. The interface between Ubc9 and RanBP2 might be unique as it is not conserved from the other types of E3 ligase. Therefore, more structural determinations of Ubc9 and E3 ligases might be required for targeting the E2-E3 interface.

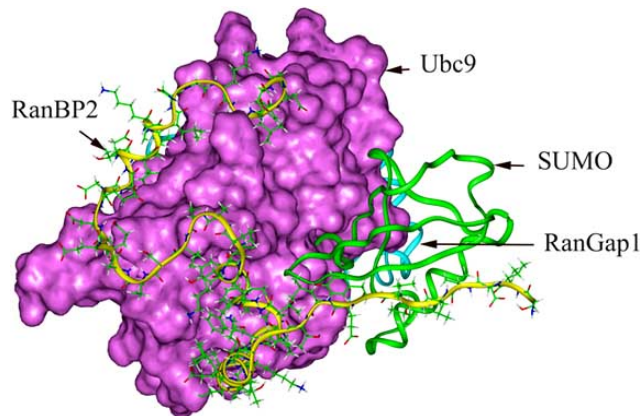


Fig. (6). Ubc9 and E3 ligase RanBP2 interaction. There are large interfaces between Ubc9 and RanBP2.

CONCLUSION

An increasing number of proteins have been identified as being modified by SUMO and associated with tumorigenesis. The analysis of the interface between Ubc9 and its interacting partners will provide the guidelines for drug virtual screening against E2 conjugating enzyme Ubc9 so that we will be able to target sumoylation regulation as a new approach to cancer therapy. The identified residues in the binding sites we have highlighted here can be targeted using a number of different virtual screening methods, such as one previously used to identify the new small molecule inhibitors for iPFK2 [25].

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REFERENCES

- [1] Palancade, B.; Doye, V. Sumoylating and desumoylating enzymes at nuclear pores: underpinning their unexpected duties? *Trends Cell Biol.*, **2008**, *18*, 174.
- [2] Baba, D.; Maita, N.; Jee, J.G.; Uchimura, Y.; Saitoh, H.; Sugawara, K.; Hanaoka, F.; Tochio, H.; Hiroaki, H.; Shirakawa, M. Crystal structure of thymine DNA glycosylase conjugated to SUMO-1. *Nature*, **2005**, *435*, 979.
- [3] Geiss-Friedlander, R.; Melchior, F. Concepts in sumoylation: a decade on. *Nat. Rev. Mol. Cell Biol.*, **2007**, *8*, 947.
- [4] Johnson, E.S. Protein modification by SUMO. *Annu. Rev. Biochem.*, **2004**, *73*, 355.
- [5] Ledl, A.; Schmidt, D.; Muller, S. Viral oncoproteins E1A and E7 and cellular LxCxE proteins repress SUMO modification of the retinoblastoma tumor suppressor. *Oncogene*, **2005**, *24*, 3810.
- [6] McDoniels-Silvers, A.L.; Nimri, C.F.; Stoner, G.D.; Lubet, R.A.; You, M. Differential gene expression in human lung adenocarcinomas and squamous cell carcinomas. **2002**, *8*, 1127.
- [7] Moschos, S.J.; Smith, A.P.; Mandic, M.; Athanassiou, C.; Watson-Hurst, K.; Jukic, D.M.; Edington, H.D.; Kirkwood, J.M.; Becker, D. SAGE and antibody array analysis of melanoma-infiltrated lymph

- nodes: identification of Ubc9 as an important molecule in advanced-stage melanomas. *Oncogene*, **2007**, *26*, 4216.
- [8] Kim, J.H.; Lee, J.M.; Nam, H.J.; Choi, H.J.; Yang, J.W.; Lee, J.S.; Kim, M.H.; Kim, S.I.; Chung, C.H.; Kim, K.I.; Baek, S.H. SUMOylation of pontin chromatin-remodeling complex reveals a signal integration code in prostate cancer cells. *Proc. Natl. Acad. Sci. U. S. A.*, **2007**, *104*, 20973.
- [9] Mo, Y.Y.; Yu, Y.; Theodosiou, E.; Rachel Ee, P.L.; Beck, W.T. A role for Ubc9 in tumorigenesis. *Oncogene*, **2005**, *24*, 2677.
- [10] Kobayashi, S.; Shibata, H.; Yokota, K.; Suda, N.; Murai, A.; Kurihara, I.; Saito, I.; Saruta, T. FHL2, UBC9, and PIAS1 are novel estrogen receptor alpha-interacting proteins. *Endocr. Res.*, **2004**, *30*, 617.
- [11] Sentis, S.; Le Romancer, M.; Bianchin, C.; Rostan, M.C.; Corbo, L. Sumoylation of the estrogen receptor alpha hinge region regulates its transcriptional activity. *Mol. Endocrinol.*, **2005**, *19*, 2671.
- [12] Giraud, M.F.; Desterro, J.M.; Naismith, J.H. Structure of ubiquitin-conjugating enzyme 9 displays significant differences with other ubiquitin-conjugating enzymes which may reflect its specificity for sumo rather than ubiquitin. *Acta Crystallogr.*, **1998**, *54*, 891.
- [13] Tong, H.; Hateboer, G.; Perrakis, A.; Bernards, R.; Sixma, T.K. Crystal structure of murine/human Ubc9 provides insight into the variability of the ubiquitin-conjugating system. *J. Biol. Chem.*, **1997**, *272*, 21381.
- [14] Bayer, P.; Arndt, A.; Metzger, S.; Mahajan, R.; Melchior, F.; Jaenicke, R.; Becker, J. Structure determination of the small ubiquitin-related modifier SUMO-1. *J. Mol. Biol.*, **1998**, *280*.
- [15] Ding, H.; Xu, Y.; Chen, Q.; Dai, H.; Tang, Y.; Wu, J.; Shi, Y. Solution structure of human SUMO-3 C47S and its binding surface for Ubc9. *Biochemistry*, **2005**, *44*, 2790.
- [16] Huang, W.C.; Ko, T.P.; Li, S.S.; Wang, A.H. Crystal structures of the human SUMO-2 protein at 1.6 Å and 1.2 Å resolution: implication on the functional differences of SUMO proteins. *Eur. J. Biochem.*, **2004**, *271*, 4114.
- [17] Reverter, D.; Lima, C.D. Structural basis for SENP2 protease interactions with SUMO precursors and conjugated substrates. *Nat. Str. Mol. Biol.*, **2006**, *13*, 1060.
- [18] Sheng, W.; Liao, X. Solution structure of a yeast ubiquitin-like protein Smt3: the role of structurally less defined sequences in protein-protein recognitions. **2002**, *11*, 1482.
- [19] Knipscheer, P.; van Dijk, W.J.; Olsen, J.V.; Mann, M.; Sixma, T.K. Noncovalent interaction between Ubc9 and SUMO promotes SUMO chain formation. *EMBO J.*, **2007**, *26*, 2797.
- [20] Reverter, D.; Lima, C.D. Insights into E3 ligase activity revealed by a SUMO-RanGAP1-Ubc9-Nup358 complex. *Nature*, **2005**, *435*, 687.
- [21] Capili, A.D.; Lima, C.D. Structure and analysis of a complex between SUMO and Ubc9 illustrates features of a conserved E2-Ubl interaction. *J. Mol. Biol.*, **2007**, *369*, 608.
- [22] Duda, D.M.; van Waardenburg, R.C.; Borg, L.A.; McGarity, S.; Nourse, A.; Waddell, M.B.; Bjornsti, M.A.; Schulman, B.A. Structure of a SUMO-binding-motif mimic bound to Smt3p-Ubc9p: conservation of a non-covalent ubiquitin-like protein-E2 complex as a platform for selective interactions within a SUMO pathway. *J. Mol. Biol.*, **2007**, *369*, 619.
- [23] Wang, J.; Hu, W.; Cai, S.; Lee, B.; Song, J.; Chen, Y. The intrinsic affinity between E2 and the Cys domain of E1 in ubiquitin-like modifications. *Mol. Cell*, **2007**, *27*, 228.
- [24] Bernier-Villamor, V.; Sampson, D.A.; Matunis, M.J.; Lima, C.D. Structural basis for E2-mediated SUMO conjugation revealed by a complex between ubiquitin-conjugating enzyme Ubc9 and RanGAP1. *Cell*, **2002**, *108*, 345.
- [25] Clem, B.; Telang, S.; Clem, A.; Yalcin, A.; Meier, J.; Simmons, A.; Rasku, M.A.; Arumugam, S.; Dean, W.L.; Eaton, J.; Lane, A.; Trent, J.O.; Chesney, J. Small-molecule inhibition of 6-phosphofructo-2-kinase activity suppresses glycolytic flux and tumor growth. *Mol. Cancer Ther.*, **2008**, *7*, 110.