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Application of Open Access Databases to determine functional connectivity between resveratrol binding protein QR2 and colorectal carcinoma

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Running Head **QR2 Function and Colorectal Cancer**

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ABSTRACT

Colorectal cancer (CRC) is a major cause of cancer-associated deaths worldwide. Recently, oral administration of resveratrol (trans-3,5,4'-trihydroxystilbene), has been reported to significantly reduce tumor proliferation in colorectal cancer patients, however, with little specific information on functional connections. The pathogenesis and development of colorectal cancer is a multi-step process that can be categorized using three phenotypic pathways, respectively, chromosome instability (CIN), microsatellite instability (MSI) and CpG island methylator (CIMP). Targets of resveratrol, including a high affinity binding protein, quinone reductase 2 (QR2), have been identified with little information on disease association. We hypothesize that the relationship between resveratrol and different CRC etiologies might be gleaned using publicly available databases. A web-based microarray gene expression data-mining platform, Oncomine, was selected and used to determine whether QR2 may serve as a mechanistic and functional biotarget within the various CRC etiologies. We found that QR2 mRNA is overexpressed in CRC characterized by CIN, particularly in cells showing a positive KRAS (Kirsten rat sarcoma viral oncogene homolog) mutation, as well as by the MSI but not the CIMP phenotype. Mining of Oncomine revealed an excellent correlation between *QR2* mRNA expression and certain CRC etiologies. Two resveratrol associated genes, adenomatous polyposis coli (*APC)* and *TP53* found in CRC were further mined, using cBio portal and Colorectal Cancer Atlas which predicted a mechanistic link to exist between resveratrol→*QR2*/*TP53*→CIN.

Multiple web-based data mining can provide valuable insights which may lead to hypotheses serving to guide clinical trials and design of therapies for enhanced disease prognosis and patient survival. This approach resembles a BioGPS, a capability for mining Web-based databases that

can elucidate the potential links between compounds to provide correlations of these interactions with specific diseases.

Key Words Oncomine; Colorectal Cancer Atlas; cBio Portal; Resveratrol; Colorectal Carcinoma (CRC); Quinone reductase 2

Oncomine is a readily available web-based platform that gives insight into cancer genomics by providing microarray datasets for different cancers (www.oncomine.org) (Rhodes et al., 2004; Rhodes et al., 2007). The expression level of the *QR2* gene in CRC was analyzed by searching the Oncomine database. The mRNA expression of *QR2* in clinical samples of normal versus CRC patients and *QR2* mRNA expression among different types of CRC were also compared. The cBio Cancer Genomics Portal (http://cbioportal.org) is a resource for exploring, visualizing, and analyzing data from multidimensional cancer genomics by reducing molecular profiles

from cancer tissues and cell lines into understandable genetic, epigenetic, gene expression, and proteomic events. The query interface thus enables researchers to interactively explore genetic alterations across samples, genes, and pathways. This database (Cerami et al., 2012; Gao et al., 2013) was used to explore the connectivity of resveratrol associated genes across all CRC studies available in the databases. The Colorectal Cancer Atlas database (http://www.colonatlas.org) is an additional open platform accessed by using categorized lists of genes, sequence variations, cell lines and techniques (Chisanga et al., 2016). The gene information page includes features such as gene details, domain details, post-translational modifications, protein identification, sequence variations, pathways and protein-protein interactions. We explored the Colorectal Cancer Atlas to interactively probe the protein-protein network of resveratrol target *QR2*. Combined with Oncomine and the cBio Cancer Genomics Portal, the Colorectal Cancer Atlas

provided the third source for a workflow platform in categorizing molecular mechanisms of resveratrol and its association with clinical outcomes found in CRC.

Resveratrol has been shown to have chemopreventive effects in CRC and its utility in adjunctive therapy has been supported by clinical studies (Nguyen et al., 2009; Patel et al., 2010). We evaluated whether the gene of its high affinity target protein, *QR2*, can be used to generate hypotheses regarding chemopreventive efficacies of resveratrol on CRC with varying etiologies. The Oncomine database revealed that *QR2* is correlated with CIN harboring the *KRAS* mutation; with statistically significant overexpression of *QR2* mRNA occurring in *KRAS*-mutated compared to wild type *KRAS* carcinomas. *KRAS* (12p12.1) encodes a GTP-binding protein that, when mutated, can cause a loss of intrinsic GTPase activity and, accordingly, elicits constitutive signaling through the RAS-RAF-MEK-ERK cascade. Elevated levels of *QR2* could conceivably increase demand for its co-substrate, N-ribosyl nicotinamide (NRN), by enhancing hydrolysis of NAD^+ or $NADP^+$. Reduction in the levels of NAD^+ or $NADP^+$ can result in an enhanced oxidized cellular environment. High expression of *QR2* could also result in increased cellular levels of oxidized NRN which can cause G1/S cell cycle arrest and inhibit inosine 5' monophosphate dehydrogenase (Gharehbaghi et al., 1994), an enzyme involved in de novo GTP biosynthesis. It is however noteworthy that an increased production of guanine nucleotides occurs in rapidly proliferating cells ultimately affecting the functioning of mutated *KRAS*. In addition, since NAD+ functions as the substrate for poly(ADP-ribose) polymerase 1 (PARP-1), a decrease in its level would diminish formation of poly (ADP-ribosylated) proteins that play key roles in several nuclear events that include DNA repair, replication, and transcription (Nakamura et al., 2003; Sauve, 2008). In a broader context, however, most studies listed (Table 1) contain insufficient details on the CIN pathway to CRC. This limits definitive evaluation of *QR2* gene

expression in the CIN pathway for conclusive statements or hypotheses to be reached. The most pronounced *QR2* changes were found in studies by Notterman et al. (2001) which showed an almost 400% increase in colon adenocarcinoma compared to healthy tissue. The study included patients from a wide age range (ages 30 to 89), from different races, early to advanced CRCs, male and female, and *TP53* mutations (Table S1). Utilizing Oncomine to distinguish *QR2* expression for each of these subgroups, we did not find correlations among *QR2* expression and any of these characteristics. By excluding CIMP-high patients in this study and observing that the CIN pathway is more commonly found in patients (Table 2), we hypothesize that these *QR2* mRNA alterations are correlated with the CIN etiology. However, the putative molecular determinants that underlie or contribute to the significant increase in *QR2* expression in adenocarcinoma remain to be determined. By contrast, as observed in the top 10% of altered genes, Skrzypczak et al. (2010) reported a decrease in *QR2* expression in epithelial adenoma compared to that in normal tissue. However, the study included only females, sporadic CRC cases and used unpaired controls. Gender differences do not play a role in *QR2* expression in CRC, since almost all of the studies identified in Oncomine distinguish between males and females, but none showed gender differences in *QR2* expression. The significance of this study is limited, however, by small sample size (10 healthy vs. 5 adenoma) and that cancerous tissue was not paired with adjacent non-involved mucosa.

The Oncomine database is available both as a subscription database and as open access. Although the free Oncomine version is very useful for generating general hypotheses regarding specific genes of interest, our analysis was limited by the paucity of gene expression data on *QR2* and the lack of classification in several studies. To further elucidate the molecular mechanisms of resveratrol and its association with clinical outcomes found in CRC, cBio portal

for Cancer Genomics (http://cbioportal.org) (Cerami et al., 2012; Gao et al., 2013) and Colorectal Cancer Atlas (Chisanga et al., 2016) were included with Oncomine, building on our recently introduced functional/activity network (FAN) analysis (Hsieh et al., 2016). Through systematic query of the publicly available databases, respectively, Oncomine, cBio portal and Colon Cancer Atlas, we propose that bioefficacy of resveratrol observed in CRC patients operates by the following pathway: resveratrol→*QR2*/TP53→CIN pathway (intersected either by *APC*↔*KRAS* or *APC*↔*PIK3CA*).

This combined Oncomine-cBio Portal-Colorectal Cancer Atlas workflow platform engenders three simple steps: (i) identify resveratrol binding target *QR2* and its association with the etiology of CRC using Oncomine (Rhodes et al., 2004; Rhodes et al., 2007), (ii) explore and verify whether genetic alterations exist for the identified resveratrol-associated genes/proteins across CRC samples in cancer genomics projects using cBio portal (Cerami et al., 2012; Gao et al., 2013) and (iii) explore Colorectal Cancer Atlas to interactively probe the protein-protein network of resveratrol target *QR2*. To our knowledge, this is a hitherto unexplored strategy that provides rationale as well as foundation in seeking certainty of biological plausibility.

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Authors' contributions

ES researched and performed the Oncomine query under supervision of TCH and wrote the first draft of the manuscript. ES received a Master's of Science in Cell Biology, New York Medical College, Valhalla, NY. TCH. ES and JMW designed the research, analyzed the data, and wrote the early drafts of manuscript. BD, JTP edited and corrected the manuscript. BBD drafted this manuscript and corresponded with Dr. John Harbell at IVA for consideration of publication of this manuscript. All authors approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C and Schultz N (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2: 401-404.

Chisanga D, Keerthikumar S, Pathan M, Ariyaratne D, Kalra H, Boukouris S, Mathew NA, Al Saffar H, Gangoda L, Ang CS, Sieber OM, Mariadason JM, Dasgupta R, Chilamkurti N and Mathivanan S (2016) Colorectal cancer atlas: An integrative resource for genomic and proteomic annotations from colorectal cancer cell lines and tissues. Nucleic Acids Res.; 44: D969-974.

Gaedcke J, Grade M, Jung K, Camps J, Jo P, Emons G, Gehoff A, Sax U, Schirmer M, Becker H, Beissbarth T, Ried T and Ghadimi BM (2010) Mutated KRAS results in overexpression of DUSP4, a MAP-kinase phosphatase, and SMYD3, a histone methyltransferase, in rectal carcinomas. Genes Chromosomes Cance; 49: 1024-1034.

Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C and Schultz N (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6: pl1.

Gharehbaghi K, Sreenath A, Hao Z, Paull KD, Szekeres T, Cooney DA, Krohn K and Jayaram HN (1994) Comparison of biochemical parameters of benzamide riboside, a new inhibitor of IMP dehydrogenase, with tiazofurin and selenazofurin. Biochem Pharmacol 48: 1413-1419.

Hong Y, Downey T, Eu KW, Koh PK and Cheah PY (2010) A 'metastasis-prone' signature for early-stage mismatch-repair proficient sporadic colorectal cancer patients and its implications for possible therapeutics. Clin Exp Metastasis 27: 83-90.

Hsieh TC, Wu ST, Bennett DJ, Doonan BB, Wu E and Wu JM (2016) Functional/activity network (FAN) analysis of gene-phenotype connectivity liaised by grape polyphenol resveratrol. Oncotarget 7(25), 38670-38680, doi: 10.18632/oncotarget.9578

Kaiser S, Park YK, Franklin JL, Halberg RB, Yu M, Jessen WJ, Freudenberg J, Chen X, Haigis K, Jegga AG, Kong S, Sakthivel B, Xu H, Reichling T, Azhar M, Boivin GP, Roberts RB, Bissahoyo AC, Gonzales F, Bloom GC, Eschrich S, Carter SL, Aronow JE, Kleimeyer J, Kleimeyer M, Ramaswamy V, Settle SH, Boone B, Levy S, Graff JM, Doetschman T, Groden J, Dove WF, Threadgill DW, Yeatman TJ, Coffey RJ, Jr. and Aronow BJ (2007) Transcriptional recapitulation and subversion of embryonic colon development by mouse colon tumor models and human colon cancer. Genome Bio 8: R131.

Kurashina K, Yamashita Y, Ueno T, Koinuma K, Ohashi J, Horie H, Miyakura Y, Hamada T, Haruta H, Hatanaka H, Soda M, Choi YL, Takada S, Yasuda Y, Nagai H and Mano H (2008) Chromosome copy number analysis in screening for prognosis-related genomic regions in colorectal carcinoma. Cancer Sci 99: 1835-1840.

Nakamura J, Asakura S, Hester SD, de Murcia G, Caldecott KW and Swenberg JA (2003) Quantitation of intracellular NAD(P)H can monitor an imbalance of DNA single strand break repair in base excision repair deficient cells in real time. Nucleic Acids Res 31: e104.

Nguyen AV, Martinez M, Stamos MJ, Moyer MP, Planutis K, Hope C and Holcombe RF (2009) Results of a phase I pilot clinical trial examining the effect of plant-derived resveratrol and grape powder on Wnt pathway target gene expression in colonic mucosa and colon cancer. Cancer Manag Res 1: 25-37

Notterman DA, Alon U, Sierk AJ and Levine AJ (2001) Transcriptional gene expression profiles of colorectal adenoma, adenocarcinoma, and normal tissue examined by oligonucleotide arrays. Cancer Res 61: 3124-3130.

Patel KR, Brown VA, Jones DJ, Britton RG, Hemingway D, Miller AS, West KP, Booth TD, Perloff M, Crowell JA, Brenner DE, Steward WP, Gescher AJ and Brown K (2010) Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. Cancer Res 70: 7392-7399.

Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, Barrette TR, Anstet MJ, Kincead-Beal C, Kulkarni P, Varambally S, Ghosh D and Chinnaiyan AM (2007) Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia.; 9: 166-180.

Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, Barrette T, Pandey A and Chinnaiyan AM (2004) ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia 6: 1-6.

Sabates-Bellver J, Van der Flier LG, de Palo M, Cattaneo E, Maake C, Rehrauer H, Laczko E, Kurowski MA, Bujnicki JM, Menigatti M, Luz J, Ranalli TV, Gomes V, Pastorelli A, Faggiani R, Anti M, Jiricny J, Clevers H and Marra G (2007) Transcriptome profile of human colorectal adenomas. Mol Cancer Res 5: 1263-1275.

Sauve AA (2008) NAD+ and vitamin B3: from metabolism to therapies. J Pharmacol Exp Ther 324: 883-893.

Skrzypczak M, Goryca K, Rubel T, Paziewska A, Mikula M, Jarosz D, Pachlewski J, Oledzki J and Ostrowski J (2010) Modeling oncogenic signaling in colon tumors by multidirectional analyses of microarray data directed for maximization of analytical reliability. PLoS One 5.