Quality control & quality assurance of canine biological specimens available through the Pfizer-CCOGC biospecimen repository for comparative oncology studies

Rachael Thomas 1,3, Mark Simpson 1, Hiroyuki Mochizuki 1, Christina Williams 1, Kelsey Poomran 1, Katie Kennedy 1, Christina Mazzuco 1, Jaime F. Modiano 2,4 and Matthew Breen 1,2,5

1. Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC; 2. Center for Comparative Medicine and Translational Research, North Carolina State University, Raleigh, NC; 3. Laboratory of Cancer Biology and Genetics, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; 4. Department of Veterinary Clinical Sciences, University of Minnesota, St. Paul, MN; 5. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA

The Canine Comparative Oncology and Genomics Consortium (CCOGC) was conceptualized in 2004 to reflect growing interest in parallel studies of canine and human cancer genomics. In 2007 the CCOGC incorporated as a ‘not for profit’ entity with 501(c)(3) status, with a lead gift from Pfizer Animal Health and substantial donations from the American Kennel Club Canine Health Foundation and Morris Animal Foundation.

The primary aims of the CCOGC are:
1. To develop opportunities arising from a more advanced understanding of the genetics and biology of companion animal cancers to guide the development of novel technologies, and to permit the integration of appropriate canine cancers in the global study of cancer biology and therapy.
2. To establish a tumor histology and collaborations across diverse disciplines in canine and comparative cancer biology, and aid sharing of resources and reagents to develop a high-utility biospecimen repository to support these aims.

In 2007, the CCOGC initiated a collection of biospecimens from naturally-occurring primary canine cancers across all dog breeds in eight major veterinary institutions within the USA.

To date the Pfizer-CCOGC Biospecimen Repository has received 69,356 specimens from almost 2,000 canine cancer patients (Figure 1) across seven major cancers that represent the greatest impact on canine health and welfare, with maximum comparative and translational relevance.

This report describes a quality control and assurance assessment performed on the Pfizer-CCOGC Biospecimen Repository prior to releasing specimens to the scientific community in October 2012.

Pfizer-CCOGC biospecimen collection procedures

All specimens contained with the repository are collected using standardized institutionally approved protocols with informed owner consent, prior to initiation of chemotherapy.

The following specimens are collected from each case and are processed within one hour:
- tumor tissue (snap-frozen, and both formalin- and OCT-frozen)
- healthy tissue (snap-frozen, and both formalin- and OCT-frozen)
- peripheral blood

Each submission is associated with the following patient data:
- age
- weight
- tumor diagnosis
- breed
- gender
- tumor subcategory
- neuter status
- collection date
- pathology report

Specimens are deidentified at source, divided into representative replicates as required, barcoded and recorded in a central, real-time ‘Tissue Tracker’ relational database along with associated clinical history, prior to transfer to the Biospecimen Repository and housed within the National Cancer Institute (NCI) Frederick Central Repository Services.

Study aims

This internally commissioned study was performed to evaluate the quality of the Pfizer-CCOGC Biospecimen Repository prior to commencing sample withdrawal by the scientific community.

Quality control and assurance parameters were assessed on a panel of biospecimens distributed randomly across the eight submitting institutions and the seven tumor histologies represented in the repository:

1) To perform a detailed pathology review of the tumor histology of selected biospecimens.
2) To assess the quantity and yield of nucleic acid obtained from tumor tissue and peripheral blood, as the primary reagents destined for downstream comparative genomics studies.

Pathology re-review of biospecimens

A total of 331 formalin-fixed and paraffin-embedded (FFPE) tumor specimens, representing a cross-section of all seven tumor histologies, were subjected to rigorous pathology re-review by a panel of board-certified veterinary pathologists at the NCI.

H&E-stained specimens were 1) evaluated in context with the original histologic diagnosis assigned by the submitting veterinarian, and 2) assessed for tumor versus stromal contamination by routine histopathology, immunohistochemistry and quantitative morphometry.

Outcome of pathology evaluation

The proportion of cases for which the original diagnosis was corroborated ranged from 64% (hemangiosarcoma) to 100% (lymphoma and osteosarcoma), with a mean of 89% across all cases. An alternate diagnosis was reached for 9% of specimens, and the remaining 5% of specimens showed no clear evidence of a neoplastic process. The low level for hemangiosarcoma was due primarily to lack of sufficient tumor tissue in the submitted specimen to permit conclusive diagnosis.

All 564 biospecimens were processed successfully to generate a quantifiable nucleic acid specimen, for which a yield and quality score could be assigned (summarized in Figure 4).

Acquisition of biospecimens for nucleic acid assessment

A total of 188 cases were selected at random from the biospecimen repository, providing proportional representation of each tumor histology and submitting institution (Figure 3).

Outcome of nucleic acid quality assessment

Across the panel of 188 cases > 73% of all DNA samples, and 64% of all RNA samples met the criteria for classification as optimal quality samples (category A). The proportion of samples classified as suboptimal (category B) was highest for tumor RNA (18.5%), followed by tumor DNA (15.1%), tumor RNA (6.5%), and tumor DNA (6.5%).

Across the panel of 188 cases > 73% of all DNA samples, and 64% of all RNA samples met the criteria for classification as optimal quality samples (category A). The proportion of samples classified as suboptimal (category B) was highest for tumor RNA (18.5%), followed by tumor DNA (15.1%), tumor RNA (6.5%), and tumor DNA (6.5%).

Conclusions

Quality control and assessment of randomly selected samples from the Pfizer-CCOGC Biospecimen Repository, representing approximately 10% of all cases submitted, demonstrated:

1) The diagnostic classification of almost 85% of submitted specimens was corroborated by in-depth re-review by a panel of independent veterinary pathologists.
2) The proportion of specimens yielding nucleic acid considered to be of suitable quality for most downstream genomics applications (categories A and B) was 89.4% for tumor DNA, 52.1% for tumor RNA and 99.5% for blood DNA.

This evaluation of the Pfizer-CCOGC Biospecimen Repository provides further data to reinforce the potential value of this sample collection for furthering advances in canine and comparative cancer studies.

The repository is now open for access to the scientific community through a peer-reviewed application procedure. Almost 1,500 specimens have been released for research studies to date.

For further details contact the CCOGC via http://www.cccog.net or administration@cccog.net.

References

1. http://www.cccog.net/
2. Webster JD et al., J Biomol Tech. 2011 September; 22(3): 108–118

Acknowledgements

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