for ubiquitination assay instruction; and S. Sakiyama for reading the manuscript.

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Comprehensive genomics linking between neural development and cancer: neuroblastoma as a model

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Abstract

Cancer cells are derived from their precursor cells, which normally develop to the matured cells to form individual organs. Neuroblastoma, one of the most common pediatric solid tumors, originates from possible cancer stem cells derived from the neural crest. During the development, neural crest cells segregate into several lineages such as sensory, enteric and sympathetic neurons. However, the genetic events to cause neuroblastoma occur only in the sympathetic precursor cells or cancer stem cells. Furthermore, spontaneous regression of a subset of neuroblastoma found in patients under one year of age mimics a developmentally programmed neuronal cell death that occurs in normal sympathetic neurons during the perinatal period. Thus, the genetic events to cause neuroblastoma may be programmed to occur in a lineage-specific as well as developmentally regulated manner. In this review, we discuss about the molecular link between neural development and the genesis of neuroblastoma based on our comprehensive genomics approach.

Keywords: Comprehensive genomics; Neuroblastoma cDNA project; Neural development

1. Introduction

Neuroblastoma (NBL) is one of the most common childhood cancers and is originated from the sympathetic precursor cells derived from the neural crest [1]. The clinical behavior of NBL is unique and enigmatic: the tumor cells spontaneously regress in patients under one year of age by undergoing differentiation and/or apoptosis, whereas the tumor often grows aggressively and eventually kills the patient when it occurs after one year of age [2,3]. It is thus mysterious why NBL regress or grow in an age-dependent manner. In 1963, Beckwith and Perrin have reported an interesting observation that only one of 40 microscopic fetal neuroblastomas has later become symptomatic, sporadic tumors. The remaining tumors disappeared spontaneously, suggesting that one of 250 (0.4%) fetuses has an 'in situ neuroblastoma' [4]. This observation may at least in part explain the result of NBL mass-screening performed in infants at the age of six months in Japan [5], because most of those tumors regress or take a favorable clinical course without decreasing the incidence of aggressive NBLs which are usually found in

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the patients over one year of age [6,7]. These have suggested that regression of NBL, which mimics the developmentally regulated 'programmed cell death (PCD)' of neurons [8,9], is still able to occur for about 12 months after birth. However, this idea cannot simply explain why NBLs found in the patients over one year of age do not regress.

It is highly possible that the molecular mechanism of normal development of sympathetic neurons is closely related to the regulation of NBL biology. Some important molecules of NBL, such as MYCN [10] and Trk [11], are already known to be the key players to regulate maturation of neural crest cells during development. However, we still miss a large number of genes or molecules playing important roles in the regulation of NBL biology and its genesis. In this review, we introduce a current knowledge about the molecular link between NBL and normal development of sympathetic cells, and discuss the future approaches to further understanding of the enigmatic tumor, NBL.

2. Neuronal lineage and oncogenic events

The oncogenic events to cause NBL appear to be strictly regulated and lineage-dependent, because NBL never occurs from the neural precursors of other than sympathoadrenal cell lineage (Fig. 1). The cell fate determination may be regulated by multiple transcription factors and their target genes which may include those of growth factors and their receptors [12,13]. The possible candidate genes to decide the direction of sympathetic differentiation include a human homolog of *Drosophila Acaete-Scute* proneural gene (hASH1) [14,15] and *Phox2a* [16,17] and *Phox2b* [18] of homeodomain genes. However, the precise mechanism is still elusive.

Another aspect of neuronal lineage specificity of the oncogenic events is shown by the mutation pattern of the *Trk* family genes [19]. *Trk* genes are specifically or preferentially expressed in neuronal tissues and cells. Nevertheless, the oncogenic *Trk* genes derived from translocation or somatic mutation have been exclusively observed in human malignancies with non-neuronal origin (colon cancer, papillary thyroid cancer, and acute myeloid leukemia). On the other hand, the expression levels of prototype Trk regulate the biology in cancers originated from neuronal precursor cells (neuroblastoma, medulloblastoma, thyloid C-cell hyperplasia and medullary thyroid cancer). Therefore, it should be interesting to know how the precursor cells choose the way to cause or regulate the cancer in a lineage-specific manner, that is absolutely unclear at this time.

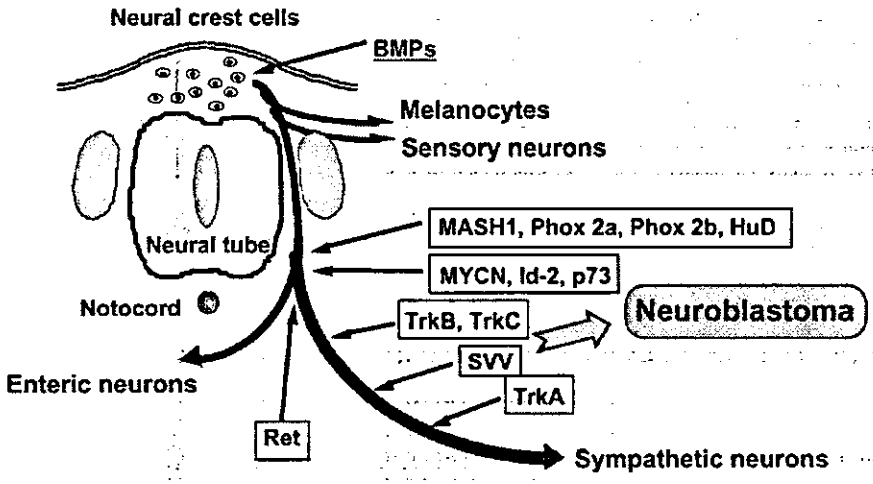

Fig. 1. Developmental lineages derived from the neural crest cells and the genesis of neuroblastoma.

3. Neural crest development and the molecules related to neuroblastoma

Neuroblastomas express many genes that are involved in the regulation of neural crest development [20–22]. The bone morphogenetic protein (BMP) signals regulate early stage of neural crest cell migration and differentiation before the decision to enter the sympathetic lineage [23], from which NBL exclusively arises. Of interest, the BMP signal is still functioning in many NBL cell lines because the treatment of the cells with BMP2 induced both phosphorylation of Smad1 and neurite outgrowth (Y. Nakamura, unpublished data). This suggests that NBLs still possess the ability to respond to the ligands that have worked at the early stage of normal neural development. Similarly, Delta/Notch signaling appears to function in NBLs by regulating neurite outgrowth [24]. Hypoxia, which induces dedifferentiation in NBL cells, decreased the expression of Notch1 [25].

hASH1 continues to be expressed at high levels in many neuroblastomas [26,27]. It is normally down-regulated after transient expression during the development of neural crest cells, that in turn promotes differentiation to the mature sympathetic neurons [28]. Targeted disruption of mouse homolog MASH1 has demonstrated the absence of sympathetic neurons [29], suggesting the important role of MASH1 expression in deciding the direction of sympathetic differentiation. Of interest, hASH1 is down-regulated during the NBL differentiation induced by retinoic acid [26,27]. hASH1 also directly represses expression of PACE4, a mammalian subtilin-like proprotein convertase that activates transforming growth factor (TGF)-β-related proteins such as BMPs [30]. This repression may shut off the BMP signaling and other factors in NBL cells. Expression of HES-1, a neuronal basic helix-loop-helix protein, represses hASH1 expression and leads the NBL cells to the status of de-differentiation [31]. As reported previously, MYCN targets Id-2 to induce its expression and the induced Id-2 inhibits the Rb tumor suppressor [32]. The still unidentified tumor suppressor(s) residing at the distal region of short arm of chromosome 1 might also be involved in this regulation because the allelic loss of the region is well correlated with amplification of *MYCN*[33,34]. The homeodomain transcription factors, Phox2a and Phox2b, are also essential for differentiation of noradrenergic neurons [35]. They may play a role in regulating the biology of NBL.

Other transcriptional regulators, that affect both neural crest development and NBL, include MYCN [36], hypoxia-inducible factor 1 (HIF-1) [37] and the tumor suppressors p53 [38] and p73 [39]. MYCN is frequently targeted to amplify in aggressive NBLs [40,41]. Since the importance of MYCN in NBL is invaluable, it is precisely discussed by M. Schwab in the separate chapter of this special issue.

4. NGF family signaling and the role of p53 and p73 in neuroblastoma

The downstream regulators of neural crest cell differentiation include neurotrophic factors and their receptors. Expression of neurotrophin receptors, TrkA and TrkB tyrosine kinases, strongly affects the biology of NBL [2,9]. TrkA, a high-affinity receptor for nerve growth factor (NGF), is expressed at high levels in NBLs of the patients with favorable prognosis, whereas it is extremely down-regulated in the tumors of those with unfavorable outcome [8,42–44]. In contrast, TrkB, a high-affinity receptor for brain-derived neurotrophic factor (BDNF), is preferentially expressed in aggressive NBLs especially with *MYCN* amplification [45]. The NBLs with high expression of TrkA frequently regress spontaneously by undergoing neuronal apoptosis as well as differentiation [8]. On the other hand, the NBLs with low expression of TrkA and amplification of *MYCN* often kill the patients. In such tumor cells, BDNF and TrkB promote tumor cell growth and metastasis in an autocrine manner [46]. Thus, the neurotrophic factors and their receptors, that normally regulate terminal differentiation of neural crest cells, may manipulate the differentiation and survival of the NBL cells [19].

Recent investigations have revealed that p53 and its family member p73 play a pivotal role in the developmentally regulated PCD of sympathetic neurons in mice [47]. The *p73*-deficient mice show neurological and immunological defects [48]. ΔNp73,

an N-terminally truncated form of p73 lacking transactivation ability, is predominantly expressed in developing brain and sympathetic neurons in mice and inhibits the neuronal PCD by blocking the proapoptotic function of p53 [47]. This observation has suggested p53, p73 and ΔNp73 to be the key regulators in the determination of neuronal differentiation and apoptosis. Interestingly, we and other investigators have recently found that p73 directly target *ΔNp73* for its expression by binding to the *ΔNp73* promoter. The induced *ΔNp73* then physically interacts with both p73 and wild type p53 to inhibit their function [49,50]. These relationships have suggested the presence of a negative feedback regulation of TAp73 by its target ΔNp73 in modulating cell survival and death of sympathetic neurons as well as NBL cells. c-Myc has also been involved in this regulatory system [51]. Since it is well recognized that the PCD of sympathetic neurons is strongly regulated by NGF signaling, elucidation of the downstream regulatory mechanism of the signaling by p53, p73 and ΔNp73 may become more important than ever. This may also help to understand the NBL biology regulated by NGF/TrkA signaling (Fig. 2). In NBL, the chromosome 1p36 region, to which p73 is mapped, is frequently deleted in advanced stage tumors. Furthermore, ΔNp73 is expressed at higher levels in unfavorable NBLs than in favorable ones [52]. In addition, according to the accumulating evidence, p73 may be one of the key factors of neural stem cells. Therefore, p73 could also be an important gene linking between neural development and cancer.

5. Comprehensive genomics of neuroblastoma

The rapid progress of the human genome project has enabled us to challenge the dynamic approaches to understanding of molecular genetic as well as cellular mechanism of cancers. It has also opened the door to unveil the molecular mechanism of neural development and neurogenic cancers. In NBL, the first trial to collect a large number of genes expressed in the CHP134 cell line was performed by K. Matsubara's group in Japan more than 10 years ago [20]. The cDNAs thus identified were published in 'Bodymap', the human gene expression database (http://bodymap.ims.u-tokyo.ac.jp/), including the information on gene expression in various tissues or cell types. Recently, R. Versteeg's group in Holland has introduced the method of serial analysis of gene

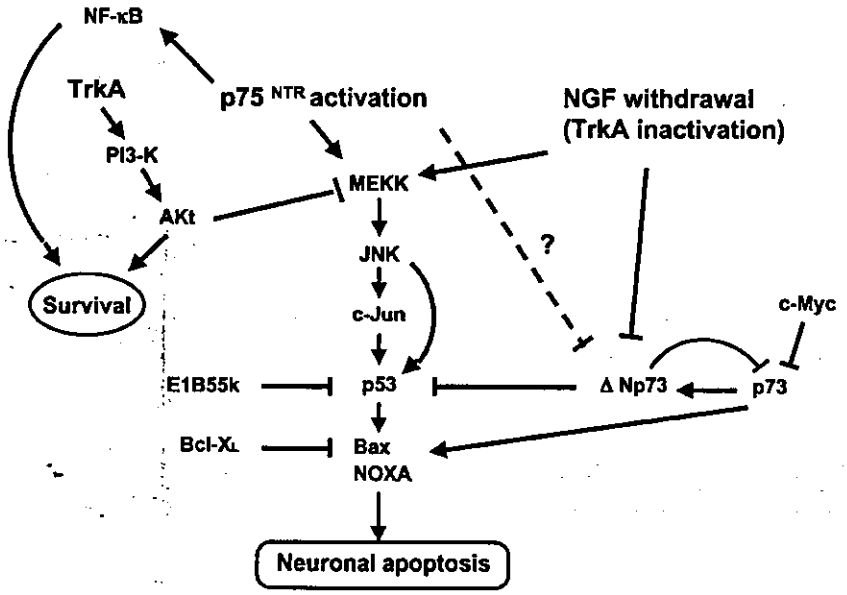

Fig. 2. The role of p53, p73 and ΔNp73 in intracellular signaling of neuronal survival and apoptosis mediated by NGF and its receptors.

Table 1
Summary of the neuroblastoma cDNA project

Number of the total clones	9,729
Number of the gene clusters	6,252
Differentially expressed genes between favorable and unfavorable subsets	
Function, known	255/1,325 (19%)
Function, unknown	502/2,115 (24%)
Total	757/3,440 (22%)
Nervous system-specific genes	156/2,297 (7%)

expression (SAGE) to identify the genes expressed in primary NBLs and cell lines in a large scale [53]. They have successfully found MEIS as an amplified gene in NBL and also identified the *MYCN* target genes [54]. Thus, the SAGE procedure is powerful for identifying the already known genes. However, it is not suitable for the approach to determine the novel genes whose functions are elusive. Therefore, we have decided to identify the individual genes expressed in the typical subsets of primary NBLs by starting the 'NBL cDNA project' which directly clones the expressed genes in a large scale from the NBL oligo-capping cDNA libraries [21,22]. In this review, we briefly present the results of and discuss about 6,252 gene clusters identified from the screening of 9,729 clones randomly picked up from three different NBL cDNA libraries: three favorable (F: stage 1, high expression of *TrkA* and a single copy of *MYCN*), three unfavorable (UF: stage 3 or 4, decreased levels of *TrkA* expression and amplification of *MYCN*) and a typical stage 4s (4s: high expression of *TrkA* and a single copy of *MYCN*) NBL libraries. This is the extended result of the previous report [22] that analyzed 4,243 cDNA clones randomly picked up from the F and UF NBL cDNA libraries.

5.1. A large scale cloning of the expressed genes from different subsets of primary neuroblastomas (Neuroblastoma cDNA project)

We randomly obtained 2,410, 2,244 and 5,075 cDNA clones from F, UF and 4s cDNA libraries and successfully sequenced the both or either end of 2,134, 2,109 and 5,004 clones, respectively (Table 1). We identified an extremely high number of 2,115 genes with unknown function compared with that (8%) found in the genes obtained from the cDNA libraries of childhood hepatoblastomas (HBLs) made by the same procedure (unpublished data). Interestingly, the average size of the genes obtained from NBLs (neuronal) was significantly larger than that from HBLs (hepatic) with an obvious tendency. We screened the genes for differential expression between F and UF NBLs by using semi-quantitative RT-PCR (16 F and 16 UF NBL samples) and found that 255 out of 1,325 known genes as well as 502 out of 2,115 novel genes were differential. In addition, RT-PCR analysis for expression in multiple human tissues showed that 156 of 2,297 genes, most of which were novel, displayed specific expression in neuronal tissues.

5.2. Expression profile and identification of the differentially expressed genes among the subsets

The expression profile of known genes was very different among the three subsets of NBL. The F subset frequently expressed neuronal specific genes including those related to neural differentiation, synapse, catecholamine metabolism, and protein degradation. On the other hand, the UF subset expressed many genes related to cell cycle control, protein synthesis and transcriptional regulation. The 4s tumor just before starting rapid regression also showed an extremely unique expression profile. It contained many apoptosis-related genes (*Bcl-10, NIP1, NIP3L, BAG-1, BID, FADD*, etc.), oncogenes (*c-Abl, c-Fos, K-Ras, c-Raf*, etc.), the other tumor-related genes (*TrkA, TGF-β, EXT2, LEU5, TNFR1, TRAP-1, ING-1, TRAIL*, etc.) and HLA family members that might be derived from the infiltrated lymphocytes into the tumor. Since the number of the genes cloned in our system was so small as compared to that of the SAGE method, we decided to examine whether or not the individual gene was expressed differentially between F and UF NBLs by using semi-quantitative RT-PCR.

The significance of the 757 differentially expressed genes was strongly implicated in understanding of NBL biology. Surprisingly, most of the genes were expressed at higher levels in F than UF subset [22]. The genes highly expressed in F subset contained those related to neuronal differentiation, migration, cell–cell interaction, protein degradation, synaptic

vesicles, catecholamine metabolism and intracellular signaling. Most of them define the neuronal-specific phenotype and maintain the neuronal function. They also included heat shock proteins and ubiquitin/proteasome-related molecules that might sense the stress. On the other hand, only about 10% of the differential genes were expressed at high levels in UF subset. The protein products of such known genes contained many transcriptional and translational regulators including oncoproteins. Notably, downregulation of dopamine-β-hydroxylase (DBH) and monoamine oxidase (MAO), the genes involved in the regulation of catecholamine metabolism, in UF as compared to F tumors could explain the previous observation that aggressive NBLs with *MYCN* amplification were dopaminergic [55].

5.3. Screening of the genes regulated by NGF in the newborn mouse SCG neurons

It is well accepted that both membrane receptors for NGF, TrkA and p75, are highly expressed in the F subset of NBL whereas they are strongly downregulated in the UF tumors with *MYCN* amplification [8,9]. The spontaneous regression only occurs in F type of NBLs, suggesting that the molecular mechanism of NBL regression is closely related to that of the PCD occurring in the late embryonic stage of sympathetic neurons. Indeed, the F type NBL expresses high levels of TrkA and p75 receptors but only a trace amount of NGF which might be supplied from the stromal cells such as Schwannian cells and fibroblasts within the tumor tissue [9]. The possible hypothesis is that only the tumor cells, which have obtained the limited amount of NGF, could survive and differentiate to ganglion-like mature cells, whereas the most cells, which have not been able to get enough amount of NGF to survive, might die. This attractive hypothesis prompted us to examine whether the differentially expressed genes between F and UF change their expression levels during the NGF-induced differentiation and the NGF-depletion-induced apoptosis in the newborn mouse superior cervical ganglion (SCG) neurons which are extremely sensitive to NGF for survival and death [56]. Among 353 genes we selected from the novel genes with differential expression, we could find 234 mouse counterparts, of which 181 primer pairs worked. They were subjected to screen for the change of expression by using semi-quantitative RT-PCR. Interestingly, seven and six genes were up- and down-regulated after NGF-induced differentiation, respectively, while one and 35 genes were up- and down-regulated after NGF depletion-induced apoptosis, respectively. Eight genes were up-regulated during NGF-induced differentiation and subsequently down-regulated after depletion of NGF, whereas 12 genes were down-regulated during the differentiation and then up-regulated after induction of apoptosis. However, 112 genes did not show any change in their expression in this system. The further analyses of those genes should give important insights into understanding of the differentiation and regression of NBL.

5.4. Chromosomal mapping of the differentially expressed genes

NBL is a cancer with extensive genomic aberrations [33,34]. According to the previous investigations, it is evident that the developmentally important genes like *MYCN* are targeted to cause NBL. Therefore, in order to identify more genes related to the NBL genesis, we mapped the 719 differentially expressed genes to human chromosomes (Fig. 3). It is obvious that there are some clusters of the differential genes in many loci on the chromosomes. The gene density mapped to each chromosome is also not the same. The genes receiving epigenetic regulations such as methylation and acetylation could also be included. The indicated mapping may give some hints for the future researches identifying important genes of NBL as well as those functioning in neural development.

5.5. cDNA microarray and array comparative genomic hybridization (array CGH)

We have recently developed our own cDNA microarray for investigation of NBL biology and mechanism of neuronal development. As many important genes of transcriptional regulator are involved, it may be useful to understand the complicated neural network during the normal

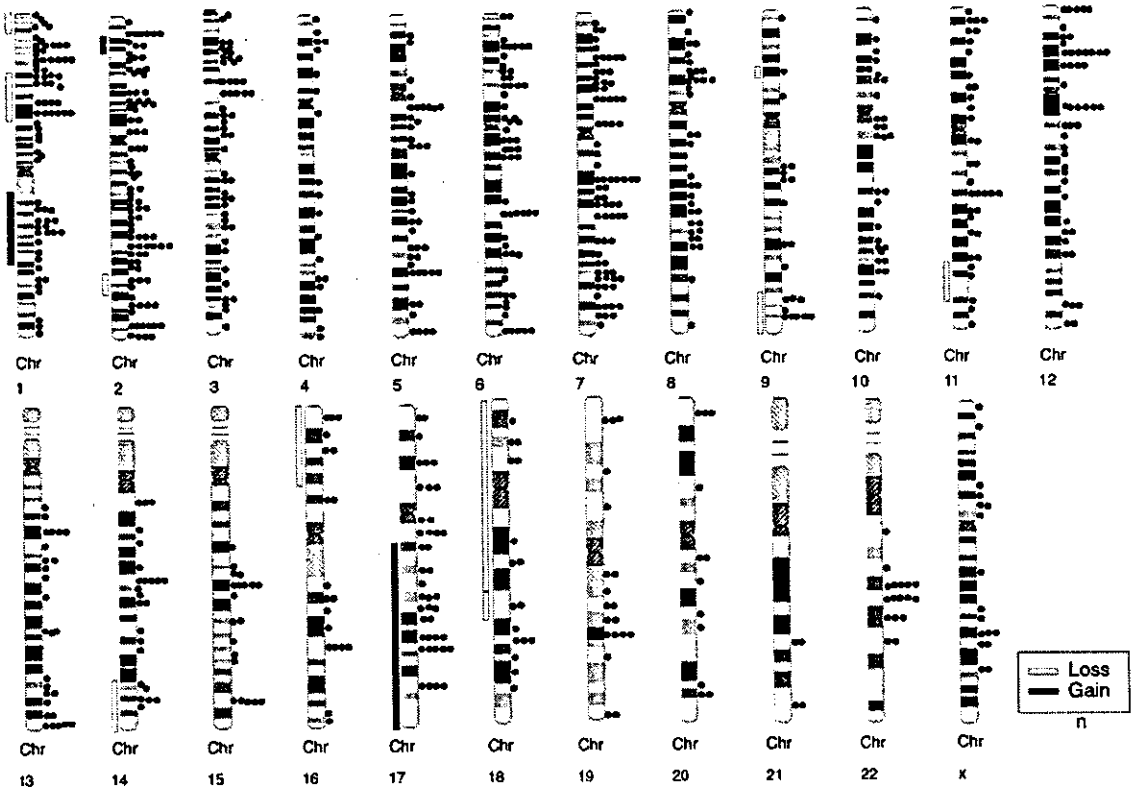

Fig. 3. Chromosomal mapping of the genes differentially expressed between favorable and unfavorable subsets of neuroblastoma. The gray and black lines indicate the regions of loss of heterozygosity (LOH) and those of chromosomal gain, respectively.

development as well as its aberrations in cancers such as NBL. Furthermore, the NBL cDNA microarray should be important for the clinical use to develop it as a diagnostic tool. The comparative and parallel studies between the results obtained from the cDNA microarray analysis and the array comparative genomic hybridization (array CGH) [57] might also be very helpful to identify the genes and to understand the molecular biology of NBL.

6. Developmental clock and the genes of neuroblastoma

Our NBL cDNA project has provided us with tremendous information about the genes expressed in different subsets with characteristic biology. Though the project is still ongoing, a temporary view so far obtained suggests the presence of a kind of rule in the expression patterns of the subset-specific genes. Fig. 4 shows the groups of genes expressed along the time axis of sympathetic neuron development. During the early stages of development, many transcription factors and their regulators may play important roles in deciding the direction of differentiation as well as in regulating cell growth and survival of neural crest-derived cells. It is interesting that many genes highly expressed in unfavorable NBLs contain transcription factors and the components of their complexes. They involve MYCN and Id family transcription factors that link to the regulation of Rb and p53 and regulate cell growth and apoptosis. The basic helix-loop-helix transcription factor, hASH1, is constitutively activated in NBL, and, by collaborating with Phox2a and Phox2b, it may regulate the arrest of

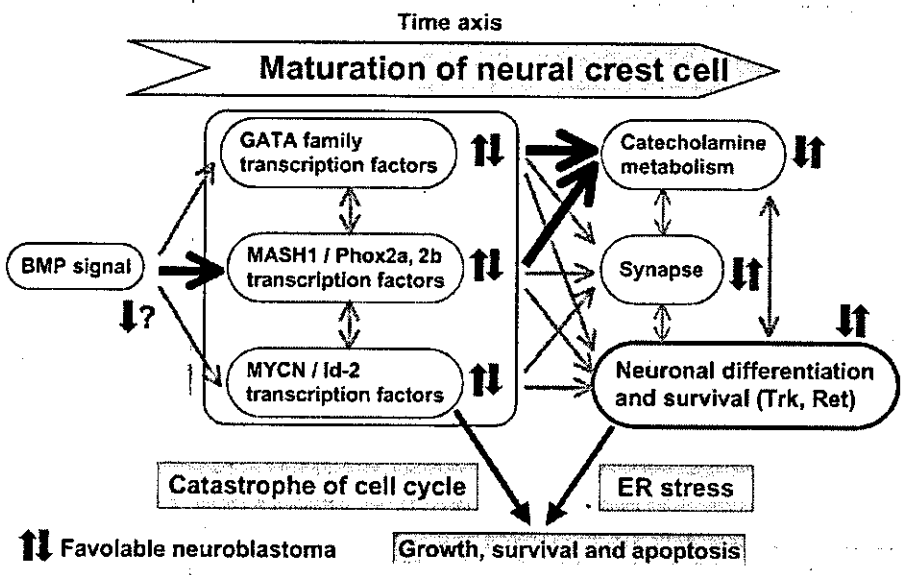

Fig. 4. The developmental time axis and the gene expression cascade during maturation of the neural crest cells. Many transcription factors are up-regulated in the unfavorable neuroblastoma, whereas the genes related to the terminal differentiation of neuron are up-regulated in the favorable neuroblastoma. ER: endoplasmic reticulum.

differentiation in unfavorable NBLs (T. Kuno et al., unpublished data). Our NBL cDNA project has also revealed that there may exist a neuronal cassette of GATA transcription factor complex that controls growth and differentiation of sympathetic progenitor cells. Some molecules in this complex are up-regulated in unfavorable NBLs (M. Aoyama et al., manuscript in preparation). Thus, many important components in the transcriptional regulators appear to be highly expressed in unfavorable NBLs and function to regulate the tumor cell growth or the status of de-differentiation.

On the other hand, a remarkable number of the genes expressed at high levels in favorable NBLs seem to encode the molecules that are necessary to maintain the neuronal function of the matured neuronal cells. They may be needed to maintain catecholamine metabolism, synapse formation, neuronal cell survival, etc. We have also identified many genes related to the ubiquitin-proteasome pathway and heat shock proteins in favorable NBLs, suggesting that they might be involved in induction of apoptosis triggered by endoplasmic reticulum (ER) stress [58].

Thus, our NBL cDNA project which is still on-going has clearly demonstrated that favorable NBL arrests its differentiation at late stage of neural development, whereas unfavorable NBL arrests at the early and immature stage.

7. From comprehensive to functional genomics of neuroblastoma aiming at the drug discovery

All genes and their products are no doubt necessary to accomplish embryonal differentiation and to maintain homeostasis of the whole human body. However, aberration of even one gene can cause disease or cancer, albeit many cancers including NBL have multiple genetic abnormalities. Therefore, we need to try to select the truly important genes that

affect the genetics and biology of NBL as key regulators.

During the functional analyses of the novel genes we selected for the last three years, several genes have been reported as important regulators especially in the neuronal system. They include Nogo (Nbla00271) [59], a negative regulator of axonal guidance, FOG2 (Nbla03139) [60], a cofactor of the GATA transcription factor complex, small GTPase RAB6B (Nbla00086) [61], Mlt1 (Nbla00106) [62], homeotic regulator homolog MAB21 (Nbla00126) [63], GTP-binding protein RAB3C (Nbla00494) [64], neurexophilin (Nxph1, Nbla00697) [65], RAB3 efffector protein RIM1 (Nbla00761) [66], cell recognition molecule Caspr2 (Nbla00831) [67], endothelin converting enzyme-like 1 (ECEL1, Nbla03145) [68], doublecortin- and calmodulin kinase-like 1 (DCAMKL1, Nbla10919) [69] and aczonin (Nbla11270) [70]. Our current analyses of the individual genes selected also suggest that the present cDNA resources derived from the primary NBLs contain many novel genes involved in NBL, neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis, and other diseases. The progress of the functional genomics of the NBL genes may lead to the discovery of the molecular targets for the therapeutic strategy.

8. Conclusion

The enigmatic tumor, neuroblastoma, is changing not to be enigmatic, because the molecular bases are now rapidly being unveiled. Indeed, the clinical and biological enigma of NBL may suggest a tight link between the cancer and the development of neural crest cells from which NBL is derived. The comprehensive genomics and future proteomics approaches may accelerate further understanding of the biology of NBL which in turn helps to clarify the molecular mechanism of normal neural development. It is important and interesting that the key regulators during the normal neural development are usually the targets of the NBL genesis. We need to intensively search for those target genes which could also be important for the drug discovery leading to the cure of the patients.

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Web-based delivery of medical multimedia contents using an MPEG-4 system

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Summary Moving picture expert group compression standard version 4 (MPEG-4) is a standard for video coding aimed at multimedia applications. MPEG-4 was developed to enable high compression rate in a low bitrate transmission via the Internet or mobile telecommunications. Although these characteristics of MPEG-4 are suitable for telemedicine, little is known about the possibility of using this technology in the field of telemedicine. We evaluated the quality of MPEG-4-encoded medical video streams and compared them with original analogue videos and audio–video-interleave (AVI) files. Although MPEG-4 video streams have the advantage of small file size, they were found to be inferior to original videos and AVI files in terms of smoothness of motion pictures, sharpness of images and clearness of sound. Illegibility of characters was a major probelem in MPEG-4 files. The score for total impression of MPEG-4 files was significantly lower than those for AVI files. The results of this study suggest that the quality of MPEG-4-encoded video streams is not adequate for telemedicine.

1. Introduction

Telemedicine is defined as the delivery of health care and sharing of medical knowledge over a long distance using a telecommunication system [1,2]. Telemedicine has great potential as a resource for medical education [3,4], remote medicine [5] and emergency medical services [6,7]. Since medical information consists of enormous amounts of data such as audio and video data, effective image coding and compression (encoding) are needed for storage and transmission of the information.

Moving picture experts group compression standard version 4 (MPEG-4) is a technology for compressing audio, video and related control data and is one of the MPEG international standards [8–10]. Unlike the former international coding standards such as MPEG-1 and MPEG-2, MPEG-4 enables a high compression rate in low and middle ranges of bitrate. The MPEG-4 system includes various tools for error robustness that improve the performance in error-prone transmission channels. Application areas of MPEG-4 technology include digital television, mobile phones, personal digital assistance (PDA) and streaming video over the Internet, which are suitable media for telemedicine. However, high compression rates of audio and video

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files results in deterioration of the quality of such files.

Little is known about the possibility of using MPEG-4 technology in the field of telemedicine [5–7]. In this study, we therefore evaluated the quality of audio and video streaming files that were made using MPEG-4 technology.

2. Materials and methods

2.1. System configurations (Fig. 1)

Analogue videos on VHS tapes were converted to digital files and compressed to MPEG-4 formats on Windows NT Server 40 SP6 (Microsoft Co. Inc., Seattle, WA)-based Flora 370 (Hitachi Co. Inc., Tokyo, Japan) computer having a 500 MHz Pentium III processor, 512 megabyte (MB) of RAM, HMVC-3000 video card (Hitachi Co. Inc.) and ample disk space. The computer was connected to 100 Base-TX Ethernet networks of our hospital in order to be used as a video streaming server. The commercial software Videonet IV Basic version 2 (Hitachi Co. Inc.) was used for digitizing, encoding and streaming of MPEG-4 video files according to the manufacturer's instructions. Briefly, video encoding was based on MPEG-4 video simple profile (ISO/IEC14496-2) [11], and the transport rate of the video stream was 192 kbps. The number of video frames per second was 15, and the size of output video was 320 (width) × 324 (height) pixels. Audio encoding was based on MPEG-1 Audio Layer II (ISO/IEC11172-3) [12], and the sampling mode of the audio files was 44.1 kHz mono. The audio and video stream data were transported on the network protocol transmission control protocol and internet protocol (TCP/IP) to client computers that were connected to networks of our hospital. The steaming video data were browsed using Internet Explorer version 5.5 (Microsoft Co. Inc.) with a plug-in MPEG-4 viewer that were provided by the software program Videonet IV. The same analogue videos were converted to audio–video interleave (AVI) formats using software DVgate version 2.5 (Sony Co. Inc., Tokyo, Japan) according to the manufacturer's instructions. Video coding was based on the National Television System Committee (NTSC) digital video format. The Number of video frames per second was 29.97, and the size of output video was 720 (width) × 480 (height) pixels. The sampling mode of the audio was 48.0 kHz mono. The AVI files were browsed using Windows Media Player version 6.0 (Microsoft Co. Inc.). As client computers, we used Windows NT Workstation 4.0-based 98MATE MA50J computers (NEC Co. Inc., Tokyo, Japan) that each had a 500 MHz Pentium III processor and a 15 in. 1024 (width) × 768 (height) pixel flat panel monitor.

2.2. Videos

Four original videos on VHS tapes were prepared for experiments (Fig. 2). They included videos of children's first-aid, emergency neurosurgery, risk management seminars and medical statistics lectures. The videos for emergency neurosurgery and medical statistic lectures contained many still pictures such as presentation slides. The duration of each video was less than 3 min.

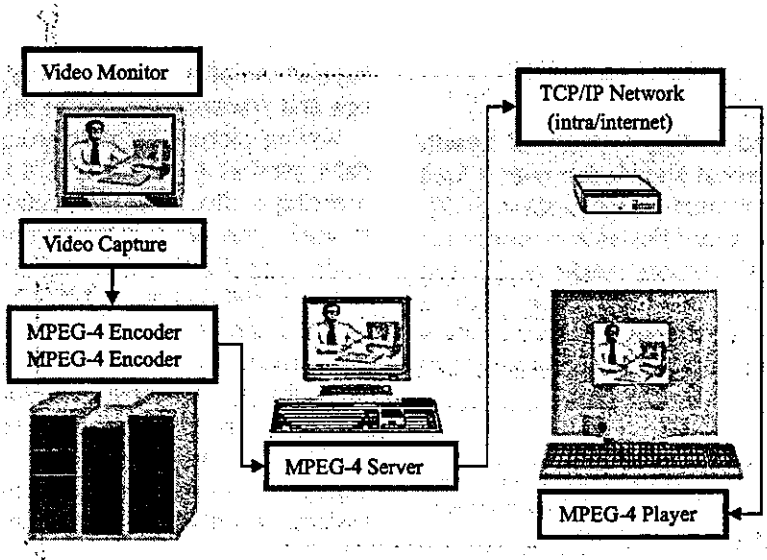

Fig. 1. Outline of the multimedia delivery system using an MPEG-4 system.

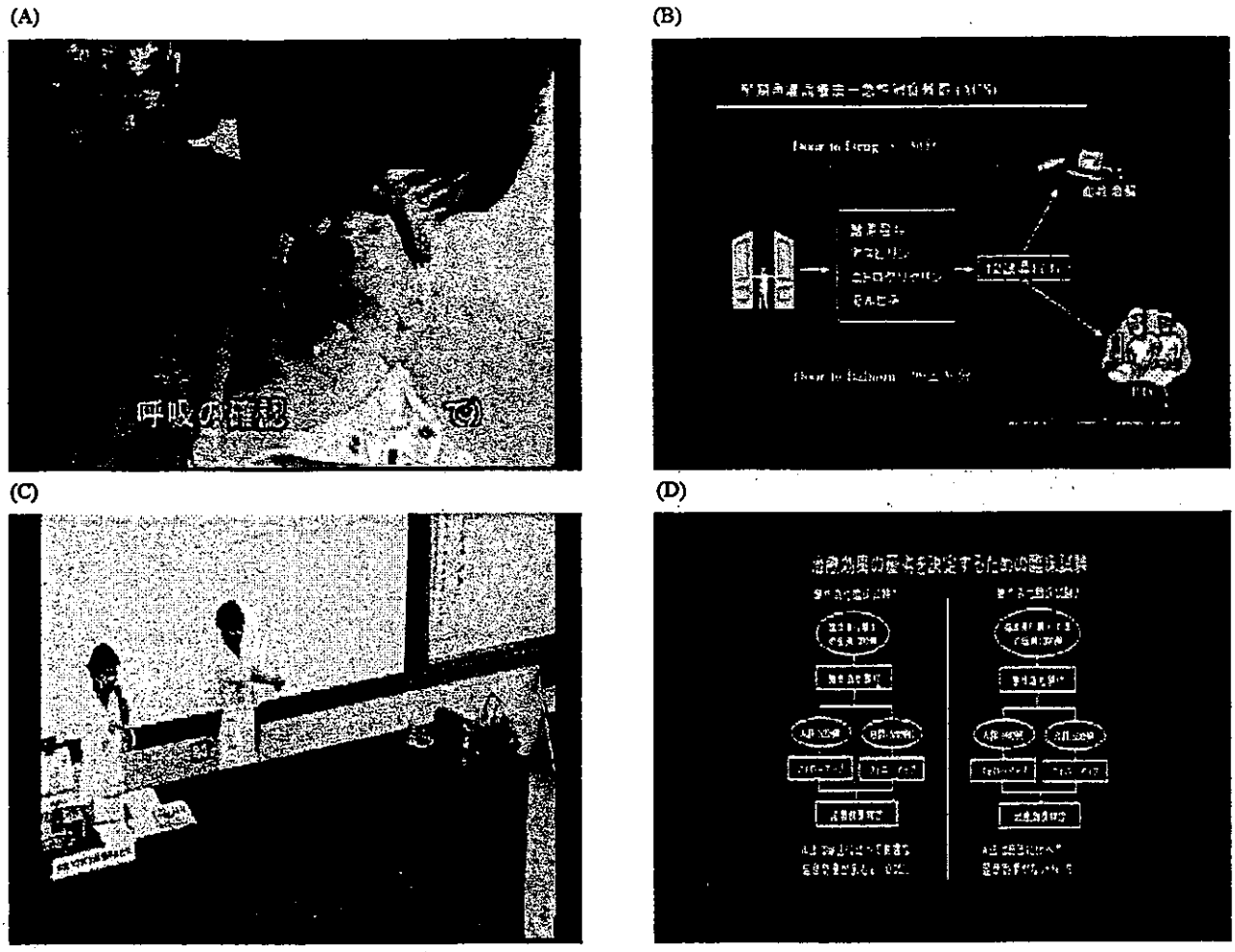

Fig. 2 Frames of MPEG-4-encoding streams: children's first-aid (A), emergency neurosurgery (B), risk management seminars (C), and medical statistics lectures (D).

2.3. Evaluation of the quality of videos

Three doctors and six nurses in our hospital subjectively judged the quality of MPEG-4-encoded video files in comparison to original analogue videos and AVI-encoded video files. The viewers firstly watched the analogue video on standard TV type monitors and then two types of steaming video files on 98MATE MA50J computers. The judgments were made in a standard testing laboratory in which the illumination of the room, brightness of monitors, sound volume and physical location of the viewers were kept fixed during the experiments. The evaluation scale was a 10 cm line that corresponds to numerical ratings between 0 and 5. The viewers evaluated the subjective quality of videos using the scales with the assistance of associated word indicators. These indicators broke the continuous scale into five equal 20-mm sections that corresponded to "excellent" (4.1–5.0), "good" (3.1–4.0), "fair" (2.1–3.0), "poor" (1.1–2.0) and "bad" (0–1.0). The evaluation items were smoothness of motion pictures, legibility of characters, sharpness of images, clearness of sound and total impression compared to those of original analogue videos.

2.4. Statistical analysis

Statistical significance of differences between two groups was tested by Wilcoxon's signed rank test. Two-way ANOVA was used for comparisons between groups and for evaluating each item described above using the computer program BMDP Statistical Software (University of California Press, Los Angeles, CA).

3. Results

3.1. File size and streaming delay

The average file size of AVI-encoded videos was 782.9 MB, whereas the average size of MPEG-4-

Table 1 File size and streaming delay

Scene		File size Mbyte	Streaming delay sec
Children's first-aid	MPEG4	5.6	4.3
	AVI	685.2	–
Emergency neurosurgery	MPEG4	23.7	3.4
	AVI	769.0	–
Risk management seminars	MPEG4	18.1	3.6
	AVI	695.7	–
Medical statistics lectures	MPEG4	21.7	3.6
	AVI	981.7	–
Average	MPEG4	17.3	3.7
	AVI	782.9	–

encoded videos was 17.3 MB (Table 1). The average streaming delay of MPEG-4-encoded videos was as short as 3.7 s.

3.2. Smoothness of motion pictures

Results of two-way ANOVA showed that the evaluation score for the degree of smoothness of motion pictures in MPEG-4 video files was significantly lower than that in the corresponding AVI video files ($P < 0.001$, Table 2).

3.3. Legibility of characters

The degree of legibility of characters in still pictures such as presentation slides was low in both MPEG-4 and AVI files. Results of two-way ANOVA showed that the illegibility of characters was significantly more prominent in MPEG-4 files ($P < 0.001$). All nine viewers judged MPEG-4 video files as "poor" or "bad" in terms of sharpness of images because of the illegibility of characters in presentation slides.

3.4. Sharpness of images

Results of two-way ANOVA showed that the evaluation score for the sharpness of images in MPEG-4 video files was significantly lower than that in the corresponding AVI video files ($P < 0.001$).

3.5. Clearness of sound

Results of two-way ANOVA showed that evaluation score for the clearness of sound in MPEG-4 video files was significantly lower than that in AVI video files ($P < 0.001$). However, the results of Wilcoxon's signed rank test showed that deterioration of sound clearness was not remarkable in two MPEG-4 video streams.

3.6. Total impression of MPEG-4 video files compared to that of original analogue video files

Results of two-way ANOVA showed that the evaluation score for the total impression of MPEG-4 video files was significantly lower than that of AVI video files ($P < 0.001$).

Table 2 Evaluation of streaming videos

Scene		Smoothness of motion pictures	Legibility of characters	Sharpness of images	Clearness of sound	Total impression compared to original videos
Children's first-aid	MPEG4	2.6 ± 0.8†	–	1.7 ± 0.6†	2.7 ± 1.1*	1.7 ± 0.4†
	AVI	4.0 ± 0.6	–	3.7 ± 0.4	3.4 ± 0.8	3.1 ± 0.8
Emergency neurosurgery	MPEG4	–	0.9 ± 0.5*	1.7 ± 0.7	2.1 ± 1.0	1.3 ± 0.5†
	AVI	–	1.8 ± 0.4	2.1 ± 0.5	3.1 ± 0.7	2.1 ± 0.5
Risk management seminars	MPEG4	2.0 ± 0.7*	–	1.7 ± 0.7†	2.0 ± 0.9	1.6 ± 0.1†
	AVI	3.3 ± 1.0	–	2.6 ± 0.8	3.2 ± 0.7	2.7 ± 0.4
Medical statistics lectures	MPEG4	–	0.8 ± 0.5†	1.3 ± 0.7*	1.7 ± 0.7†	1.2 ± 0.5†
	AVI	–	1.8 ± 0.3	1.8 ± 0.6	3.7 ± 0.8	2.1 ± 0.8
Total	MPEG4	2.3 ± 0.8†	0.8 ± 0.5†	1.6 ± 0.7†	2.1 ± 1.0†	1.4 ± 0.5†
	AVI	3.6 ± 0.9	1.8 ± 0.4	2.6 ± 0.9	3.3 ± 0.8	2.5 ± 0.8

* $P < 0.05$ as compared with AVI files.
† $P < 0.01$ as compared with AVI files.
† $P < 0.001$ as compared with AVI files.

4. Discussion

In the present study, we made web-based video streams for medical applications using the MPEG-4 system. There are several standards for Internet transmission of video streams that have been used for medical application [13], including RealMedia [4,14–16], Windows Media [3], QuickTime [17–19], MEPG-1 [20–23] and MPEG-2 [24]. According to previous reports, these formats enable a high degree of compression while retaining reasonable image quality. However, there are some limitations in the transmission of medical information using these formats through Internet and mobile communications. The algorithm used in these formats to compress audio and video data into a specific bandwidth limits the encoding bitrate that can be used. For example, the bandwidth of an MPEG-1 stream is about 1.5 Mbps and that of MPEG-2 is about 20 Mbps. In addition, the central processing units of portable devices used in mobile telecommunications limit the number of video frames per second. On the other hand, the MPEG-4 system enables transmission at a high compression rate in a bitrate as low as 5 kbps to a maximum of 5 Mbps. Thus, transmission via the Internet or intranet environments or via mobile telecommunications is possible by using the MPEG-4 system. The high compression rate in MPEG-4 encoding is suitable for streaming under these conditions. Our results showed that MPEG-4-encoded videos are satisfactory in terms of size.

The average streaming delay between requesting a media stream and playing it on the client computer was short in the case of MPEG-4 streaming videos. Video streaming via a network requires buffering of data in the client computer at the beginning of playout in order to cope with network congestion [8]. However, pre-buffering is responsible for the major part of the streaming delay. Our results showed that the steaming delay of MPEG-4 files was short enough not to get tired of waiting for playout.

High compression rates of audio and video files results in deterioration of the quality of MPEG-4-encoded streaming files. Our results showed that the quality of the MPEG-4 streaming videos encoded in the setting of our experiments was inferior to that of AVI-encoded video files and original analogue videos. The deterioration in the quality of MPEG-4-encoded videos is likely to be due to the low encoding bitrate, narrow bandwidth and low frame rate. Although the streaming configurations in our experiment are widely used in current telecommunications, the development of technology such as third-generation mobile communication and an asymmetric digital subscriber line (ADSL) will enable improvement in the quality of streaming videos by increasing the bandwidth. The higher the encoding bitrate is, the clearer and smoother the streaming images are. Assessment of the quality of MPEG-4 streaming media using the new technology is needed.

Deterioration in the quality of MPEG-4-encoded videos that contained many still images was remarkable. MPEG-4 streaming videos were inferior to AVI videos in terms of legibility of characters in still images such as presentation slides. One method for improving the quality of still images in the MPEG-4 system is to use multilayer encoding that enables to be dealt with various kinds of media such as audio, video and text-based information [8–10]. However, video streaming using multilayer encoding is not widely used at present in the field of telemedicine. Since seamless integration of various information accomplished by an MEPG-4 system is suitable for transmission of medical information, future studies should be carried out to evaluate streaming media using multilayer encoding.

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Development and evaluation of a teleradiology and videoconferencing system

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Summary

We developed a teleradiology system linking a general hospital on Sado Island to tertiary care hospitals in Niigata City. The island is 40 km from Niigata City on the mainland and has only one diagnostic radiologist (for 72,000 islanders). Fibre optic cables between Sado Island and Niigata City were used for transmission. The introduction of the teleradiology system facilitated diagnostic and therapeutic consultation with specialists in Niigata City. The performance of the system was evaluated (on a scale of 0–6, with higher scores indicating better performance) by five diagnostic radiologists, who rated 32 features of the system twice, once in April 2002 and once in September 2003. The performance ratings improved from 1.38 to 2.86. While many of the initial problems with the software had been resolved, many still remained.

Introduction

Many small hospitals in Japan are equipped with modern diagnostic imaging equipment, such as computerized tomography (CT) and magnetic resonance imaging (MRI) scanners[1]. However, there are fewer diagnostic radiologists per head of population in Japan than in the US and Europe. Therefore, diagnostic radiology expertise is scarce in rural areas and especially on Japan's isolated islands[2,3].

Sado Island is 40 km from Niigata City on the mainland. It takes about 2.5 h to get there by ferry. The island's population numbers 72,000 and is rapidly ageing. There are six hospitals on the island, with a total of 1008 inpatient beds; the largest is the Sado General Hospital (SGH). However, there is only one diagnostic radiologist on the island. For radiology cases that are difficult to diagnose, the films have to be sent from the island to hospitals in Niigata City for interpretation. Teleradiology could improve the speed and accuracy of diagnostic radiology on the island[4–6].

We have developed a teleradiology system that connects the SGH to two tertiary care hospitals in Niigata City, via a fibre optic network. The aim of the present study was to determine its usefulness and to identify problems.

Methods

System hardware

We used existing fibre optic cables between the mainland and the island (Fig 1). The fibre optic cables can transmit information at a maximum speed of 2.0 Mbit/s in an asynchronous transfer mode (ATM), at an effective bandwidth of 1.7 Mbit/s. The network linked three hospitals: the SGH, the Niigata Cancer Center Hospital (NCCH) and the Niigata University Hospital (NUH).

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